Dynamics of brain states and cortical excitability in paroxysmal neurological conditions.

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Declaration of own work

I, Prisca Bauer, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated and referenced in the thesis.

Signature:

Date:

1st of October 2016

Abstract

Epilepsy and migraine are neurological conditions that are characterised by periods of disruption of normal neuronal functioning. Aside from this paroxysmal feature, both conditions share genetic mutations and altered cortical excitability. People with epilepsy appear to be diagnosed with migraine more often than people without epilepsy and, likewise, people with migraine seem to be diagnosed with epilepsy more often than people without migraine. Changes in cortical excitability may help explain the pathophysiological link between both conditions, and could be a biomarker to monitor disease activity. In this thesis, the association between migraine and epilepsy and their relation to cortical excitability is further explored. A meta-analysis of previous population based studies provides epidemiological evidence for the co-occurrence of migraine and epilepsy. The combination of computer modelling with human electroencephalographic recordings offers insight into multi-stability of brain states in epilepsy. Results described in this thesis show that Transcranial Magnetic Stimulation can be used to measure cortical excitability, but that its use as a biomarker of disease activity in epilepsy is limited due to large interindividual variability. By combining Transcranial Magnetic Stimulation with electroencephalography, two novel variables that may contribute to cortical excitability are investigated: phase clustering, which possibly reflecting functional neuronal connectivity, and the non-linear residual of a stimulus-response curve, which may reflect brain state multi-stability. The results presented in this thesis suggest that the higher propensity to global synchronisation is not shared between epilepsy and migraine. These new variables have potential value to differentiate people with epilepsy, but not people with migraine, from normal controls.

Table of contents

Declaration of own work 2

Abstract 3

Acknowledgements 6

Publications associated with the work in this thesis 8

Posters and oral presentations 9

Contributions of the author 10

List of abbreviations 11

List of tables 12

List of figures 13

1. Aims and outline 15

2. Introduction and review of the literature 17

2.1 The association between epilepsy, migraine and headache 17

2.2 Measuring cortical excitability in epilepsy 37

3. Meta-analysis of the co-occurrence of migraine and epilepsy 64

3.1 Introduction 64

3.2 Methods 65

3.3 Results 68

3.4 Discussion 77

4. Understanding the postictal state in epilepsy through computational modelling 80

4.1 Introduction: brain states in epilepsy 80

4.2 Methods 84

4.3 Results 91

4.4 Discussion 100

5. The topographical distribution of epileptic spikes in juvenile myoclonic epilepsy with and without photosensitivity 104

5.1 Juvenile myoclonic epilepsy 104

5.2 Methods 105

5.3 Results 109

5.4 Discussion 111

6. Measuring synchronisability and multistability with TMS in healthy controls, Juvenile Myoclonic Epilepsy and Migraine 115

***Hypotheses, participants and methods* 115**

6.1 Hypotheses and aims 115

6.2 Participants 118

6.3 Stimulator and EMG and EEG recordings 119

6.4 Stimulation protocols 120

6.5 Data analysis 122

7. Calculating the resting motor threshold & assessing cortical excitability with paired-pulse protocols. 128

7.1 Participants 128

7.2 Validation of motor threshold calculation in healthy controls 131

7.3 Paired-pulse protocols in healthy controls and people with JME 133

7.4 Comparison with existing literature and critical re-appraisal 136

7.5 Discussion 142

8. Measuring epileptogenicity with TMS-EEG 145

8.1 Phase clustering measured with TMS-EEG 145

8.2 Non-linearity measured with TMS-EEG 148

8.3 Distinction of JME using non-linearity and phase clustering 150

8.4 Exploration of the topographical distributions of relative phase clustering index and non-linearity 150

8.5 TMS evoked potential analysis 154

8.6 Spatial phase distribution 154

8.7 Discussion 157

9. Summary, conclusions and context 161

9.1 Summary 161

9.2 Applicability of the findings 163

9.3 Limitations 164

9.4 Future directions 165

9.5 Conclusion 167

10. References 169

Appendix 190

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Combining work and private life can be challenging, especially when one is passionate about one’s work. I am tremendously fortunate to have a loving husband, a supporting family and fantastic friends who help me to keep a healthy balance.

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Publications associated with the work in this thesis

Chapter 2.1: PR Bauer, JA Carpay, GM Terwindt, JW Sander, RJ Thijs, J Haan, G Visser. (2013) *Headache and Epilepsy.* Curr Pain Headache Rep 17(8):351

Chapter 2.1: PR Bauer, GH Visser, GM Terwindt, MJL Perenboom, J Haan (2015) Migraine en epilepsie, twee kanten van dezelfde medaille? Epilepsie, periodiek voor professionals (Dutch) 13(2), 8-12

Chapter 2.2: PR Bauer, S Kalitzin, M Zijlmans, JW Sander, GH Visser. (2014) *Cortical excitability as a clinical marker of epilepsy: a review of the clinical application of Transcranial Magnetic Stimulation.* Int J Neural Syst 24(2):1430001

Chapter 3: MR Keezer, PR Bauer, MD Ferrari, JW Sander. (2015) *The comorbid relationship between migraine and epilepsy: A systematic review and meta-analysis.* Eur J Neurol, 22(7):1038-1047

Chapter 4: PR Bauer, RD Thijs, RJ Lamberts, DN Velis, GH Visser, EA Tolner, JW Sander FH Lopes da Silva, SN Kalitzin. *Dynamics of convulsive seizure termination and postictal generalised EEG suppression.* Under review at Brain, October 2016

Chapter 4: SN Kalitzin, PR Bauer, RJ Lamberts, DN Velis, RD Thijs, FH Lopes da Silva. *Automated video detection of epileptic convulsion slowing as a precursor for post-seizure neuronal collapse.* Accepted for publication in Int J Neural Syst, 2016

This publication is based on the data presented in chapter 4. I was involved in data collection and analysis, interpretation of the findings and writing of the manuscript.

Chapter 5: PR Bauer, K Gorgels, W Spetgens, NEC van Klink, FSS Leijten, JW Sander, GH Visser, M Zijlmans. *Interictal EEG patterns in people with Juvenile Myoclonic Epilepsy with and without photosensitivity.* Accepted for publication in Clinical Neurophysiology, 2016

Chapter 6 and 7: PR Bauer, RM Helling, C Kraan, JW Sander, GH Visser, SN Kalitzin. *Automated detection of the resting motor threshold in Transcranial Magnetic Stimulation.* Manuscript in preparation

Chapter 6 and 8: PR Bauer, RM Helling, MJL Perenboom, FH Lopes da Silva, EA Tolner, MD Ferrari, JW Sander, GH Visser, SN Kalitzin. *Phase clustering transcranial magnetic stimulation-evoked EEG responses in juvenile myoclonic epilepsy and migraine.* Manuscript in preparation

Posters and oral presentations

Chapter 4: PR Bauer, S Kalitzin, F Lopes da Silva, DN Velis, RJ Lamberts, JW Sander, RD Thijs *"Dynamics of convulsive seizure generation, termination and recovery."* Poster presentation at the 31st International Epilepsy Congress September 2015, Istanbul, Turkey.

Chapter 4: SN Kalitzin, PR Bauer, F Lopes da Silva, DN Velis, RJ Lamberts, JW Sander, RD Thijs *"Autonomous dynamics relating epileptic seizure generation, termination and postictal suppression. Model predictions and clinical validation."* Poster presentation at the 7th International Workshop for Seizure Prediction (IWSP7), August 2015, Melbourne, Australia.

Chapter 4: PR Bauer, S Kalitzin, F Lopes da Silva, DN Velis, RJ Lamberts, JW Sander, RD Thijs *"Dynamics of convulsive seizure generation, termination and recovery."* Poster presentation at the yearly meeting of the Dutch League Against Epilepsy, January 2015, Amsterdam Medical Center, Amsterdam, The Netherlands.

Chapter 4: PR Bauer, SN Kalitzin, RJ Lamberts, RD Thijs. *PGES: a neuronal brake?* Oral presentation at 11th annual meeting of Society for autonomous neurodynamics (SAND), July 2014, Instituts Pasteur, Paris, France.

Chapter 6 and 8: PR Bauer, GH Visser, R Helling, JW Sander, SN Kalitzin *"Measuring Epileptogenicity with Transcranial Magnetic Stimulation"* Poster presentation at the International Conference on Basic and Clinical Multimodal Imaging, september 2015, Utrecht, The Netherlands.

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Chapter 6 and 8: PR Bauer, SN Kalitzin, GH Visser, R Helling, JW Sander *"Cortical excitability as biomarker for Juvenile Myoclonic Epilepsy"* Oral presentation at yearly meeting of the Dutch Association for Pediatric Neurology, March 2015, Stichting Epilepsie Instellingen Nederland, Heemstede, The Netherlands.

Contributions of the author

Chapter 2: Literature review

For both literature reviews in this chapter, I gathered the literature and wrote the drafts of the manuscripts that were published.

Chapter 3: Meta-analysis

For the meta-analysis described in this chapter, conducted with Mark Keezer, I was responsible for the selection of articles to include, data extraction, interpretation of the results and co-authoring the manuscript for publication.

Chapter 4: Study 1

I contributed to the conception and design of the study, analysed the human Electroencephalography (EEG) data, and helped translating the findings of the computational model to the clinical EEG data. The computational model was made by my supervisor Stiliyan Kalitzin. I wrote the draft of the article that was submitted for publication and made a conference poster, which I presented at the International League Against Epilepsy conference in Istanbul, Turkey in September 2015. An essay describing this study was awarded the Queen Square Prize in Neurology 2015.

Chapter 5: Study 2

I initiated this study after seeing the EEG of someone with Juvenile Myoclonic Epilepsy and photosensitivity. Together with Nicole van Klink, I selected the cases included in the study and jointly supervised Koen Gorgels, 3rd year medicine student, for data extraction and analysis. Willy Spetgens re-assessed the EEG recordings. I wrote the draft of the paper that was submitted for publication together with my supervisors and Maeike Zijlmans.

Chapter 6, 7, 8: study 3

I was involved in the design and in creating the technical set-up required for the experiment. I was responsible for the recruitment and data acquisition of all participants, helped by Robert Helling. I analysed and interpreted the results under the guidance of my supervisors, and wrote the drafts of the papers that are submitted for publication as well as the conference poster. The investigation of previous studies described in chapter 7 was done jointly with Annika de Goede.

Throughout this work, I will use “I” when I, alone, was responsible for a specific task. If the task was carried out together with one or more colleagues, I will use “we”.

List of abbreviations

AED	-	Anti-epileptic Drug
aMT	-	active Motor Threshold
BECT	-	Benign Childhood Epilepsy with Centro-temporal Spikes
CSD	-	Cortical Spreading Depression
cSP	-	cortical Silent Period
EEG	-	Electroencephalography / Electroencephalographic
EMG	-	Electromyography / Electromyographic
FHM	-	Familiar Hemiplegic Migraine
GABA	-	Gamma-aminobutyric acid
ICF	-	Intracortical Facilitation
IGE	-	Idiopathic Generalised Epilepsy
GE	-	Genetic epilepsy
JAE	-	Juvenile Absence Epilepsy
JME	-	Juvenile Myoclonic Epilepsy
LICI	-	Long-interval Intracortical Inhibition
MEP	-	Motor Evoked Potential
MT	-	Motor Threshold
PGES	-	Postictal Generalised EEG Suppression
PPR	-	Photoparoxysmal Response
PR	-	Prevalence Ratio
POR	-	Prevalence Odds Ratio
rMT	-	resting Motor Threshold
SEIN	-	Stichting Epilepsie Instellingen Nederland
SICI	-	Short-interval Intracortical Inhibition
SP	-	Silent Period
SUDEP	-	Sudden Unexpected Death in Epilespy
TEP	-	TMS Evoked Potential
TLE	-	Temporal Lobe Epilepsy
TMS	-	Transcranial Magnetic Stimulation
TR/CR	-	Test Response / Conditioned Response
UMCU	-	University Medical Center Utrecht

List of tables

Table 2.1: *International Classification of Headache Disorders (ICHD)-III criteria for migraine-epilepsy syndromes.* Page 27

Table 2.2: *Key TMS measures in the context of cortical excitability.* Page 40

Table 2.3: *Influences on variables related to cortical excitability.* Page 47

Table 2.4: *TMS measures in generalised epilepsy*. Page 49

Table 2.5: *TMS measures of cortical excitability in myoclonic epilepsy*. Page 54

Table 2.6: *TMS measures of cortical excitability in focal epilepsy.* Page 55

Table 3.1: *Studies included in the meta-analysis* Page 70

Table 3.2: *Primary study data.* Page 72

Table 3.3: *Bias assessment.* Page 73

Table 4.1: *Participant characteristics.* Page 89

Table 5.1: *Group characteristics of people with JME per centre*. Page 109

Table 5.2: *Group characteristics of people with JME-PPR+ and JME-PPR*. Page 110

Table 5.3: *EEG comparison between JME-PPR+ and JME-PPR.* Page 111

Table 7.1: *Characteristics of participants with JME.* Page 129

Table 7.2: *Characteristics of participants with migraine with aura.* Page 130

Table 7.3: *Control group characteristics in studies of Badawy et al.* Page 137

Table 7.4: *Generalised epilepsy group characteristics in studies of Badawy et al*. Page 139

Table 8.1: *Median relative phase clustering index per group and stimulation modality*. Page 149

Table 8.2: *Median non-linearity per group for Transcranial Magnetic Stimulation*. Page 149

List of figures

Figure 2.1: *Transcranial Magnetic Stimulation set-up and physiology* Page 38

Figure 3.1: *PRISMA flow diagram.* Page 68

Figure 3.2: *Lifetime prevalence of epilepsy and migraine.* Page 74

Figure 3.3: *Prevalence ratio of migraine in people with epilepsy stratified by case ascertainment method.* Page 75

Figure 3.4: *Prevalence ratio of migraine in people with epilepsy, stratified by adjustment for confounders.* Page 76

Figure 3.5: *Prevalence ratio of epilepsy in people with migraine.* Page 76

Figure 4.1: *Bi-stability.* Page 82

Figure 4.2: *EEG recording with postictal generalised EEG suppression* Page 83

Figure 4.3: *Schematic representation of the computational model.* Page 85

Figure 4.4: *Output from the computational model.* Page 92

Figure 4.5: *Gamma distributions of ictal and postictal period durations in the model.* Page 94

Figure 4.6: *Relation between the interclonic interval (ICI) and PGES duration in the model.* Page 95

Figure 4.7: *Relation between connectivity, interclonic interval and PGES in the model.* Page 96

Figure 4.8: *Gamma distribution of ictal and PGES period durations in human EEG data.* Page 97

Figure 4.9: *Linear fit of the interclonic interval (ICI) in human seizures.* Page 98

Figure 4.10: *Relation between interclonic interval and PGES duration in EEG data.* Page 99

Figure 5.1: *Examples of localised and generalised EEG discharges outside intermittent photic stimulation.* Page 108

Figure 5.2: *Examples of generalised EEG discharges outside intermittent photic stimulation without a clear maximum.* Page 108

Figure 6.1: *Motor threshold calculation based on the stimulation response curve.* Page 123

Figure 7.1: *Correlation between the visually estimated threshold and computed thresholds.* Page 131

Figure 7.2: *Visually estimated resting motor threshold for all groups.* Page 132

Figure 7.3: *Calculated motor threshold for all groups.* Page 132

Figure 7.4: *Long interstimulus interval intracortical inhibition (LICI) curve in controls.* Page 134

Figure 7.5: *Long interstimulus interval intracortical inhibition (LICI) curve of four healthy controls.* Page 134

Figure 7.6: *Long interstimulus interval intracortical inhibition (LICI) curves in people with JME.* Page 135

Figure 7.7: *Comparison of my long interstimulus interval intracortical inhibition (LICI) with the literature.* Page 136

Figure 7.8: *Re-digitalised long interstimulus interval intracortical inhibition recovery (LICI) curves of the controls from studies from Badawy et al.* Page 138

Figure 7.9: *Re-digitalised long interstimulus interval intracortical inhibition (LICI) recovery curves of the JME and JAE groups from two studies from Badawy et al.* Page 140

Figure 7.10: *Long interstimulus interval intracortical inhibition (LICI) recovery curves with standard error of mean.* Page 141

Figure 8.1: *Boxplots of the relative phase clustering index (rPCI) for all groups.* Page 146

Figure 8.2: *EEG frequency band of the maximal phase clustering index.* Page 147

Figure 8.3: *Boxplots of the non-linearity of the TMS-EEG response curve.* Page 148

Figure 8.4: *Scatter plot of non-linearity and relative phase clustering index.* Page 150

Figure 8.5: *Topographical distribution of the relative phase clustering index.* Page 151

Figure 8.6: *Effect of medication (levetiracetam) on the relative phase clustering index in one participant with JME.* Page 152

Figure 8.7: *Topographical distribution of non-linearity of the TMS-EEG response curve.* Page 153

Figure 8.8: *TMS-evoked potentials.* Page 155

Figure 8.9: *Group-averaged phase distribution of maximal phase clustering index.* Page 156

Figure 9.1: *Overview of the findings of this thesis.* Page 163

1

Aims and outline

"The beginner should devote maximal effort to discovering original facts by making precise observations, carrying out useful experiments and providing accurate descriptions."[1]

The studies described in this thesis aim to increase the understanding of the changes in the functioning of the brain that lead to the epilepsy and migraine. Both conditions are *paroxysmal,* meaning that the symptoms are only apparent after a sudden transition to an attack. Studies in the past decades have linked both conditions to each other and to changes in cortical excitability. In this thesis, I investigate what cortical excitability is, and why it may play a role in the pathophysiology of epilepsy and migraine. I start by reviewing the existing literature on epilepsy, migraine and cortical excitability. Then I describe a study involving a computational model of epilepsy and human clinical electroencephalographic (EEG) recordings that shed new light on the brain state transitions in epilepsy. Computational models are a relatively recent addition to neuroscientific research that can help understand the sudden transitions that occur in the brain in paroxysmal conditions. In the second part of this thesis I describe novel variables that may be used to measure brain state instability with Transcranial Magnetic Stimulation (TMS) and EEG in people with epilepsy and migraine.

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[1] Ramón y Cajal S., (translation by Swanson N, Swanson LW). *Advice for a young investigator.* The MIT Press, Cambridge, MA, 1999, p86

Chapter 2 is a general introduction to epilepsy and migraine and an overview of the literature about the possible link between both conditions based on epidemiological, genetic and neurophysiological findings. The second part of the chapter provides an overview of the literature about cortical excitability and how this can be measured with TMS.

Chapter 3 describes a meta-analysis about the co-occurrence of migraine and epilepsy in the general population.

In **chapter 4** I show how computational modelling can help understand epilepsy and brain state transitions. After a brief introduction to computational models of epilepsy, I describe a study in which we show that findings from a computational model can be used as leading hypotheses for analysis of human EEGs. I validated the findings of the model in 48 human EEGs of convulsive seizures.

Chapter 5 describes a retrospective study of the interictal EEG pattern in people with Juvenile Myoclonic Epilepsy (JME) with and without photosensitivity. I highlight the difference in focal abnormalities between these two presentations of JME.

Chapter 6 describes the participants and methods for my study with TMS, EEG and electromyography (EMG) in JME and migraine. I included 38 healthy controls, 8 people with JME and 12 people with migraine.

In **chapter 7,** I present the results of the EMG part of the TMS study. First, I describe a new method for the computation of the resting motor threshold. Second, I show the results of paired-pulse protocols and contrast them with existing literature.

The results of the TMS - EEG experiments are shown in **chapter 8**. I demonstrate that TMS and EEG may be used to measure two essential hallmarks of paroxysmal state.

In **chapter 9,** I link the findings of the different studies described in this thesis, discuss their potential application and suggest directions for future research. I conclude with an overview of the contributions of this work to the field of epilepsy and migraine research.

2

Introduction and review of the literature

"...no inquiry should be started without having the relevant literature at hand."[2]

2.1 The association between epilepsy, migraine and headache

Epilepsy and migraine appear to share different features: the symptoms are thought to originate from the brain and in both conditions, the symptoms are paroxysmal, meaning that they are present during attacks, but between the attacks there are no apparent signs of the condition. In this chapter, I review the existing literature about epilepsy and migraine and the possible relation between both. In the second part of the chapter, I give an overview of cortical excitability, and describe its relation to epilepsy.

2.1.1 General introduction about epilepsy

Seizures are defined as *"transient occurrence of signs and/or symptoms due to abnormal excessive or synchronous neuronal activity in the brain"* (Fisher *et al.*, 2014). Epilepsy is characterised by *"an enduring predisposition to generate epileptic seizures and by the neurobiologic, cognitive, psychological and social consequences of this condition. The*

[2] Ramón y Cajal S., (translation by Swanson N, Swanson LW). *Advice for a young investigator.* The MIT Press, Cambridge, MA, 1999, p61

definition of epilepsy requires the occurrence of at least one epileptic seizure" (Fisher *et al.*, 2014). Pro-epileptogenic drugs or physiological conditions can trigger symptomatic seizures in anyone, but in the case of epilepsy, the seizures occur repeatedly and without such a trigger (Jefferys, 2010). In Europe, the estimated prevalence of active epilepsy, which means that someone is taking medication to control seizures, or had at least one seizure in the preceding year, ranges from 3.3 to 7.8 per 1000 inhabitants (Forsgren *et al.*, 2005). In the United Kingdom, epilepsy is the second most common neurological condition after stroke, with a lifetime prevalence of around 4 per 1000 for active epilepsy (MacDonald *et al.*, 2000). Seizures are more frequent, and it is estimated that around 5% of the general population will have at least one non-febrile seizure during their lifetime (Sander, 2003). The incidence of epilepsy is around 50 per 100.000 per year (Kotsopoulos *et al.*, 2002). It is highest in the first 15 years of life and peaks again at 70 years of age (MacDonald *et al.*, 2000).

Epilepsy is a paroxysmal condition. This means that there are sudden transitions between normal functioning of the brain and seizures. Seizures are associated with increased neuronal excitability and neuronal synchronisation (Devinsky *et al.*, 2013; Staley, 2015). Rather than being one single diagnosis with a clear aetiology, multiple factors on different levels contribute to the phenotype of epilepsy. First, malfunction of ion channels that regulate the membrane potential of neurons can lead to increased excitability (Gardiner, 2005; Lu and Wang, 2009; Noebels, 2015; Turnbull *et al.*, 2005). Mutations in several genes that code for (parts) of these ion channels are known to be associated with epilepsy, such as for example the α-subunit of the sodium channel that is encoded by the SCN1A gene. Mutations in this gene lead to several forms of epilepsy such as genetic epilepsy and febrile seizures (GEFS+) and the more severe Dravet syndrome that is associated with severe learning difficulties (Gardiner, 2005). Another example is the KNCQ2 gene that codes for a subunit of potassium channels. Some mutations in this gene cause benign familial epilepsy of childhood (Gardiner, 2005).

Neurotransmitters are signalling substances in the brain that influence the excitability of groups of neurons by synaptic inhibition (GABA) or excitation (Glutamate) (Casillas-Espinosa *et al.*, 2012). Neurons have specific receptors on the membrane to react appropriately to the neurotransmitters, such as opening sodium or potassium channels. Disequilibrium of neurotransmitters can be associated with epilepsy, for

example when the quantity of available neurotransmitter is altered, or when defects in the receptors cause inappropriate reactions of the neurons to the neurotransmitters (Casillas-Espinosa *et al.*, 2012; Treiman, 2001). There is increasing evidence that non-neuronal cells in the brain, such as glial cells, may play an important role in the pathophysiology of epilepsy (Devinsky *et al.*, 2013; Janigro and Walker, 2014). Glial cells help maintain the equilibrium of the extracellular space around neurons by absorbing neurotransmitters, ions and water, they mediate immunity and inflammation, and contribute to the function of the blood-brain barrier (Devinsky *et al.*, 2013). Through these different pathways, glial cells contribute to increased brain excitability and possibly to epilepsy (Devinsky *et al.*, 2013).

Neurons cooperate in networks, and are either connected directly or through synapses. Experiments combining computational models with *in vitro* preparations demonstrated that large neuronal populations are necessary to sustain epileptic activity. Tens of thousands of neurons are thought to be involved in microseizures (Jefferys, 2010). It is hypothesised that neurons that form such epileptic networks are more likely to entrain each other to oscillate in unison, creating the synchronous activity that is one of the hallmarks of epilepsy. In some forms of epilepsy, neurons can oscillate in very high frequencies, up to 300Hz, which is probably a sign of a pathological process (Engel and Lopes da Silva, 2012). It is unlikely that single neurons are able to produce these oscillations, and they are possibly caused by recurrent connectivity of pyramidal cells and direct connections between axonal membranes called "gap junctions" (Helling *et al.*, 2015). Epileptic networks can involve cortical neurons and also connect cortical neurons to deeper brain structures such as the thalamus (Lopes da Silva, 1991).

Disruption of normal neuronal functioning, which manifests as seizures, can have many causes. Like fever, it is a sign of an underlying general illness. The classification of epilepsy is of major importance for the correct treatment. Epilepsy can be classified based on the anatomical and aetiological source of the seizures. Advances in the understanding of genetics, neuroimaging and neurophysiology in the last decennia have prompted a revision of the classification of epilepsy that was proposed in 1969. In this classification, seizures could be "localisation-related" or "generalised", now seizures are either "focal", which means that they originate at some point within

networks limited to one hemisphere, or "generalised", denoting seizures that rapidly engage bilaterally distributed networks (Berg *et al.*, 2010; Fisher *et al.*, 2014; Scheffer *et al.*, 2016). The aetiological classification underwent more radical changes. The old classification distinguished "idiopathic" (presumed hereditary predisposition), "symptomatic" (caused by a known or suspected condition of the central nervous system) and "cryptogenic" (the cause is hidden or occult) epilepsy. With the advances of genetic techniques, which have revealed that many mutations could be associated with epilepsy, the term "idiopathic" became obsolete, and is now replaced by "genetic". "Symptomatic" epilepsy was replaced by "structural" and "metabolic" epilepsy, denoting a distinct structural (for example a brain tumour, or hippocampal sclerosis) or metabolic condition (for example mitochondrial disease) that is associated with an increased risk of epilepsy. The distinction between this type of epilepsy and "genetic" epilepsy may be blurred in certain cases as structural and metabolic conditions can also have genetic causes. The somewhat vague "cryptogenic" epilepsy is now replaced by the more direct word "unknown", which also includes as of yet unidentified genetic, metabolic and structural causes (Berg *et al.*, 2010). Throughout this thesis, I will use the new classification. When discussing existing previous work done by others, I will use the same terminology as in the original article.

There are several treatment options for epilepsy. The first line of treatment consists of pharmacological treatment with anti-epileptic drugs (AEDs). About 20 AEDs exist, each with different molecular targets (Brodie, 2010). Despite the development of new drugs, the percentage of people with epilepsy that respond to treatment has remained stable around 70% in the past decennia (Brodie, 2010; Brodie *et al.*, 2012). Novel pharmaceutical agents include the AMPA-receptor antagonist perampanel and the sodium channel inactivating lacosamide (Brodie, 2010). Recently, cannabidiol, a substance derived from the cannabis plant, has come to the attention of the epilepsy community after reports that children with severe epilepsy were successfully treated using this substance (Friedman and Devinsky, 2015). Approximately 30% of people with epilepsy continue to have seizures despite appropriate pharmacological treatment (Brodie, 2010; Brodie *et al.*, 2012). A small proportion of these people are eligible for surgical treatment (Jobst and Cascino, 2015). In two randomised controlled trials, epilepsy surgery was shown to lead to seizure freedom in 58% and 73% of people undergoing the procedure (Wiebe *et al.*, 2001, Engel *et al.*, 2012). The presence and

location of a lesion are factors that determine the outcome. For example, surgical treatment of lesional temporal lobe epilepsy, is more successful than treatment of non-lesional epilepsy or extra-temporal epilepsy (Jobst and Cascino, 2015). Other treatment options in refractory epilepsy include vagal nerve stimulation and the ketogenic diet (Fridley *et al.*, 2012; Klein *et al.*, 2014).

There is mounting evidence that epilepsy is not "just" a condition of the brain, but a systemic condition (Keezer *et al.*, 2016). Other conditions such as migraine, depression and cancer are associated with epilepsy (Gaitatzis *et al.*, 2012). Some of these have a direct causal relationship with epilepsy. For example, a cancer that has metastasised to the brain can cause epileptic seizures. The relationship with other conditions that often accompany epilepsy, such as depression, is still unclear. Some evidence is emerging that epilepsy and depression share common pathophysiological mechanisms linked to inflammatory responses in the brain (Vezzani *et al.*, 2011, 2013). I will show in section **2.1.3** and **chapter 3,** that migraine is common in people with epilepsy and vice versa. Both conditions share several features: they are paroxysmal, thought to arise from the brain and changes in cortical excitability may contribute to the pathophysiology. In the next paragraph, I give a short summary of what is currently known about migraine.

2.1.2 General introduction about migraine

Migraine is characterised by recurrent attacks of severe headaches, accompanied by nausea, vomiting and hypersensitivity to sensory stimuli. Worldwide, the prevalence of migraine is 10% on average, 6% in men and 14% in women (Jensen and Stovner, 2008; Stovner *et al.*, 2007). It is more prevalent in North America and Europe than in Africa and South America (Jensen and Stovner, 2008). The associated costs and disability are high (Jensen and Stovner, 2008; Olesen *et al.*, 2012). The pathophysiological mechanism of migraine is incompletely understood. For a long time it was thought that the headache in migraine was caused by vasodilatation, but several lines of evidence have demonstrated that vasodilatation is neither necessary nor sufficient to cause a migraine attack (Goadsby, 2009; Pietrobon and Moskowitz, 2012). There is now ample evidence that an imbalance between inhibitory and excitatory factors in the brain activates or sensitises the trigeminal nociceptors around the large blood vessels in the meninges. This leads to activation of second and third order central trigeminovascular neurons, which then activate brain areas involved in the modulation

of sensory information, such as the thalamus, hypothalamus, frontal cortex, anterior cingulate cortex, basal ganglia, and insula (Borsook *et al.*, 2015; Goadsby, 2009; Pietrobon and Moskowitz, 2012; Vecchia and Pietrobon, 2012). Several recent studies have suggested that a lack of habituation to repeated sensory stimuli may be a key feature that differentiates people with migraine and healthy controls (Brighina *et al.*, 2009; Coppola *et al.*, 2005; Demarquay and Mauguière, 2015). This would be in agreement with the idea that the activity of brain areas involved in sensory modulation is disrupted.

In about 30% of people, the headache attack is preceded by an aura, a sign, usually of visual nature, but it can involve other sensory modalities in some people (Goadsby, 2009). Some people only have auras and no headache (Naeije *et al.*, 2014). The neurophysiological correlate of the migraine aura is thought to be a slow wave of cortical spreading depression (CSD). So far, CSD has only been measured in animal models, where a slow wave of depolarisation was seen spreading over the occipital cortex (Leao, 1947; Leão, 1944). The speed of this wave matched the speed of the progression of visual auras in people (3mm/min) (Dreier and Reiffurth, 2015; Goadsby, 2009; Leao, 1947). Whether the aura triggers the headache or is merely an expression of abnormal processing of an otherwise normal stimulus is still matter of debate (Dreier and Reiffurth, 2015; Goadsby, 2009; Lipton *et al.*, 2010; Pietrobon and Moskowitz, 2012). The fact that only 30% of people with migraine have an aura, favours the altered sensory processing hypothesis ahead of the aura as a trigger for the headache (Goadsby, 2009). On the cellular level, CSD is characterised by depolarisation of neurons. This is not unique to migraine and with the development of invasive EEG recordings it became apparent that non-spreading depolarisation also plays a role in stroke, subarachnoidal haemorrhage and traumatic brain injury (Dreier and Reiffurth, 2015). Mutations in genes such as CACNA1A, ATP1A2 and SCN1A have been linked to familiar hemiplegic migraine (FHM), but non-FHM migraine is probably a polygenic condition (Freilinger *et al.*, 2012; Spillane *et al.*, 2015).

The treatment of migraine consists of analgesics, and for more severe cases (about 20% of cases) triptans. People who used triptans were more often referred to specialists, reflecting the more severe nature of their condition (Becker *et al.*, 2008). About 40% of people with migraine achieve full remission, another 40% have a low frequency of

attacks, but 20% continue to have regular attacks despite treatment (Jensen and Stovner, 2008). Poor prognosis is associated with a high attack frequency and early age of onset (Jensen and Stovner, 2008). Recently, it was shown that a single magnetic pulse, applied on the occipital cortex in the early phase of the aura could prevent the attack from developing in 39% of people, compared to 22% of those given a placebo stimulation (Lipton *et al.*, 2010). With the current understanding of migraine pathophysiology, it is hard to imagine how a single magnetic pulse could reverse the pathophysiological cascade leading to a migraine attack, but the device was nevertheless approved for medical use in the United States of America and in the United Kingdom in January 2014 (NICE, 2014). Like epilepsy, migraine rarely comes alone (Becker *et al.*, 2008; Buse *et al.*, 2010; Gaitatzis *et al.*, 2012; Jensen and Stovner, 2008; Le *et al.*, 2011). Interestingly, migraine and epilepsy often co-occur. In the next paragraphs, I will explore the relationship between epilepsy and migraine in-depth.

2.1.3 Comorbidity of migraine and other types of headache and epilepsy

Migraine and epilepsy are both disorders in which a, probably genetically determined, imbalance between excitatory and inhibitory factors results in spells of altered brain function and autonomic symptoms. There is convincing evidence of an association between migraine and epilepsy from epidemiologic, genetic and pathophysiologic studies. In the next section, I review recent advances in the understanding of the relation between headache (including migraine) and epilepsy with emphasis on studies published in 2012 (the word 'headache' is used interchangeably for all types of headache including migraine; when studies specifically refer to migraine, this is mentioned). I will discuss new epidemiologic studies on the co-occurrence of headache and migraine with epilepsy, and review studies on headache with a specific temporal relationship with epileptic seizures such as peri-ictal and ictal headache, including a proposal for classification. Recent findings regarding the genetic bases of migraine and epilepsy will be addressed and an attempt made to link the pathogenic effects of known migraine mutations to recent *in vivo* and *in vitro* findings. Two recent studies will be discussed in detail. One directly investigated *in vivo* differences in cortical excitability between epilepsy and migraine. The other measured cortical spreading depolarisation in people with a subarachnoid haemorrhage using subdural electrodes, convincingly linking cortical spreading depolarisation to epilepsy and migraine. In the conclusion of this section, I suggest directions for future research.

In the general population, the lifetime prevalence of headache is about 46% (Stovner *et al.*, 2007), and that of migraine 10-22% (Smitherman *et al.*, 2013; Stovner *et al.*, 2007). The prevalence of active epilepsy is 0.3-0.7% (Forsgren *et al.*, 2005; MacDonald *et al.*, 2000; Sander, 2003). Recent studies of the association of headache with epilepsy have resulted in contradictory results. Some reported incidence and prevalence rates of headache in epilepsy cohorts comparable to healthy populations (Ito *et al.*, 2004; Kwan *et al.*, 2008; Syvertsen *et al.*, 2007). It was argued that study designs and cultural influences may have lead to underreporting of headache in those studies (Kwan *et al.*, 2008). In contrast, other studies reported a higher prevalence of headache in people with epilepsy (Nunes *et al.*, 2011; Yankovsky *et al.*, 2005). Likewise, a significant association between migraine and epilepsy was reported in some older studies (Le *et al.*, 2011; Ottman *et al.*, 2011; Syvertsen *et al.*, 2007; Téllez-Zenteno *et al.*, 2005) but not in others (Brodtkorb *et al.*, 2008; Buse *et al.*, 2010). A more recent study found no association between chronic migraine and epilepsy (Chen *et al.*, 2012).

In children and adolescents, recent studies have provided less contradictory evidence of an association between migraine and epilepsy. In a population based study in the United States of America, about 7000 adolescents aged 13-18 were asked to complete a symptom-based survey on headache and self-reported history of neurologic, heart, digestive, skin and inflammatory problems (Lateef *et al.*, 2012). Based on this survey, 8% were classified to have migraine without aura and 0.9% to have migraine with aura, based on ICHD-II criteria (Olesen, 2004). Epilepsy or seizures were reported by 148 participants (2.3%). Headaches, including migraine, were weakly associated with epilepsy or seizures (OR 2.02, 95% CI: 1,04-3.94). The association between migraine and seizures was, however, not significant (OR 1.51 95% CI: 0.5-4.57) (Lateef *et al.*, 2012).

In a cross-sectional study on 400 children with epilepsy aged 3-17 years who were seen in a neurologic clinic, overall migraine prevalence based on ICHD-II criteria, was 25% (Kelley *et al.*, 2012). In children aged twelve years or younger, the prevalence was 21%, compared to 32% in adolescents (12-17 years). Benign epilepsy with centrotemporal spikes and Juvenile Myoclonic Epilepsy (JME) were more often associated with migraine than other types of epilepsy, such as absence epilepsy (Kelley *et al.*, 2012). These findings are partially in line with an earlier study that showed that people with

JME are more than four times as likely to have migraine than people without JME (Schankin *et al.*, 2011).

A population-based study that included 9500 Icelandic schoolchildren aged between six and sixteen years, reported an epilepsy prevalence of 7.7/1000 and a migraine prevalence of 11.8/100 (Baldin *et al.*, 2012). Migraine prevalence was higher in girls of twelve to sixteen years old than in boys of the same age group (19.9/100 respectively 13.5/100). One fifth of the children with epilepsy were also classified as having migraine, compared to about a tenth of the children who did not have epilepsy. Prevalence ratios were only significant in a univariate model (PR 2.02, 95% CI 1.17–3.51), before adjustment for age, occurrence of febrile seizures, moving sickness, recurrent diarrhoea, fainting spells and visual disturbances.

Recent studies in adults provided less convincing evidence of an association between epilepsy and migraine. In a study that included 200 adult subjects with epilepsy seen at a tertiary centre, migraine lifetime prevalence, according to ICHD-II classification, was 10.9% and tension-type headache 19.4% (Duchaczek *et al.*, 2013). These figures seem to be in line with the prevalence in the general population (Smitherman *et al.*, 2013). People with idiopathic generalised epilepsy (IGE, old classification) reported interictal migraine significantly more often (18%) compared to the total epilepsy cohort (11%) (Duchaczek *et al.*, 2013).

An Italian referral-based study conducted in epilepsy and headache centres included 1,200 people from 18-81 (Tonini *et al.*, 2012). Of subjects who were treated in epilepsy centres, 30% concomitantly suffered from primary headache, of which 17% was classified as migraine. Of the patients seen in headache centres 1.6% concomitantly had epilepsy. The authors suggest that there is no strong evidence for an association of primary headache and epilepsy.

In a study on genetics of epilepsy that included 730 participants, 32% of women and 15% of men also reported migraine (Winawer and Connors, 2013). The prevalence of migraine with aura was significantly increased when other family members were affected by a seizure disorder; however, this was not the case for migraine without aura. This effect was only significant for first-degree relatives (OR 2.5,CI 1.13-5.46), interpreted as a strong support for a genetic association or a common genetic predisposition to migraine and epilepsy.

In conclusion, results of older, as well as recent studies, on the co-occurrence of headache / migraine with epilepsy are contradictory. This may partly be caused by the classification of headache and migraine in various studies. Headache is a subjective complaint, whereas migraine is a condition with clear classification criteria. Despite this distinction, the terms "migraine" and "headache" (or "migraineous headache") are sometimes used interchangeably, obscuring results and confusing interpretation. Headache and migraine diagnoses are often self-reported, with limited questionnaires without validation by direct interview. Migraine, especially migraine with aura can, therefore, often be missed. To understand how often migraine and epilepsy co-occur in the same person in the general population, my colleagues and I conducted a meta-analysis of ten studies (1.5 million participants in total) that is described in **chapter 3** of this thesis.

2.1.4 Classification issues. Migralepsy and ictal headache

When looking at individual headache and epilepsy attacks, there is evidence for a time-dependent relationship between epilepsy and headache. Over the years, many attempts have been made to describe and classify cases of simultaneous headache and epilepsy. Recent developments demonstrate that this issue is not resolved, despite advanced diagnostic methods.

The International classification of headache disorders (ICHD)-III classification of 2013 defines three categories with overlap between headache and seizures: migraine-triggered seizures (so called 'migralepsy'), 'hemicrania epileptica' and 'postictal headache' (see table 2.1) (Olesen *et al.*, 2013). The ILAE classification of epilepsy does not include headache at all (Berg *et al.*, 2010). The 2013 ICHD-III classification defines migralepsy as a seizure triggered by a migraine aura fulfilling the ICHD-III criteria. The seizure should occur during or within one hour of the migraine aura and fulfil diagnostic criteria of an epileptic seizure, according to the ILAE classification (Berg *et al.*, 2010; Olesen *et al.*, 2013). It has been shown that cases of migralepsy are extremely rare and may have been confounded with occipital seizures (see below) (Verrotti *et al.*, 2011). In hemicrania epileptica, headache with migraine features lasts seconds to minutes, while the patient also has signs of a partial epileptic seizure. The headache develops synchronously with the seizure, is on the same side as the epileptiform discharge seen on the EEG recording and resolves immediately after (treatment of) the seizure (Olesen *et al.*, 2013). For the diagnoses of migralepsy and hemicrania epileptica

an EEG recording during the attack is essential. In more than half of the 35 migralepsy cases described in literature, however, ictal EEG recordings were unavailable, so that uncertainty remained as to whether the (migraine) aura triggered the seizure or whether it was part of the seizure. When clear descriptions of attacks, and EEG and neuroimaging were available, 40% of reported cases were suggestive of epileptic seizures, and not of migralepsy. Only a handful of published cases fulfilled all the criteria for migralepsy (Sances *et al.*, 2009). The same applies for hemicrania epileptica.

Table 2.1: International Classification of Headache Disorders (ICHD)-III criteria for migraine-epilepsy syndromes.

Criteria	Migraine aura triggered seizure	hemicrania epileptica	postictal headache
A	A seizure fulfilling diagnostic criteria for one type of epileptic attack, and criterion B below	Any headache fulfilling criterion C	Any headache fulfilling criterion C
B	Occurring in a patient with *migraine with aura* and during or within 1 hour after, an attack of migraine with aura	The patient is having a partial epileptic seizure	The patient has recently had a partial or generalised epileptic seizure
C	Not better accounted for by another diagnosis	Evidence of causation is demonstrated by both of the following: 1. Headache has developed simultaneously with onset of the partial seizure 2. Either or both of the following: a) Headache has significantly improved immediately after the partial seizure has terminated b) Headache is ipsilateral to the ictal discharge	Evidence of causation demonstrated by both of the following: 1. Headache has developed within 3 hrs afer the epileptic seizure has terminated. 2. Headache has resolved within 72 hrs after the epileptic seizure has terminated.
D		Not better accounted for by another ICHD-III diagnosis	Not better accounted for by another ICHD-III diagnosis

Headache is occasionally the only manifestation of an epileptic seizure and it has been argued that this should be seen as a separate entity (Belcastro *et al.*, 2011). Proposed diagnostic criteria for so called "ictal epileptic headache" include headache, located on either side and localised ictal epileptiform discharges on the EEG recording. The diagnosis requires epileptiform EEG abnormalities to be present concomitantly with the headache, which should resolve immediately upon treatment with intravenous anti-epileptic medication (Parisi *et al.*, 2012). The incidence of ictal epileptic headache is difficult to estimate, as an (ictal) EEG is not routinely performed in evaluation of

headache without other complaints, but it is probably rare. Only a few of such cases have been reported recently (Dainese *et al.*, 2011; Fanella *et al.*, 2012).

Epileptic seizures in the occipital lobe can resemble a migraine aura, as the main symptoms are visual hallucinations, illusions and reduced vision (Adcock and Panayiotopoulos, 2012). In some cases, there may be oculomotor symptoms, such as repetitive movements or tonic deviation of the eyes. The subjective visual symptoms of occipital epilepsy can particularly be confused with a migraine aura. It is essential to be aware of the differences: epileptic visual hallucinations usually occur within seconds and typically last a few minutes, whereas in a migraine aura hallucinations develop more slowly, over the course of a few minutes, and typically last 15-20 minutes. Epileptic visual hallucinations are usually coloured and circular, while the visual effects in migraine are often uncoloured and linear. To complicate matters further, more than half of the subjects affected by occipital seizures also have concomitant migraine-like postictal headaches (Adcock and Panayiotopoulos, 2012).

These classification issues demonstrate that, while the difference between headache and epilepsy is clear in most cases, it is important to be aware that this differentiation can be very challenging in certain cases. Correct differentiation and diagnosis is of paramount importance for effective treatment. With this in mind, it is advisable to perform an ictal EEG when confronted with severe recurrent headaches that are refractory to conventional treatment in order to rule out the possibility of epilepsy as the underlying cause for the headache.

2.1.5 Peri-ictal headache

Peri-ictal headache is common in people with epilepsy, where the seizure and headache follow each other in time. The pathophysiology of this phenomenon is not well understood. The headache can have migraine-like characteristics, but peri-ictal headache is, by definition not migraine, as according to the ICHD-III classification, migraine should not be attributed to another disorder (in case of an underlying disorder, it is termed “symptomatic migraine”). One of the most recent studies of peri-ictal headache included 200 subjects with epilepsy (Duchaczek *et al.*, 2013). The authors made a distinction between interictal headache (not time-locked to a seizure) and peri-ictal headache (preictal, ictal, postictal). Preictal headache was defined as headache beginning within 24 hours before the onset of a seizure if the headache ceased before the seizure or lasted until the onset of a seizure, regardless of the

headache duration. Ictal headache, occurring during a seizure, could, of course, only be assessed in people with intact consciousness during the seizure. If headache occurred immediately after the seizure or when it was present at the time when the person regained consciousness, it was termed postictal. One third (N=71) of the people with epilepsy in this study reported peri-ictal headache: preictal headache was reported by 16 people and postictal headache by 61 people, some people reported both pre- and postictal headache. Only one person reported ictal headache. In 95% of people, peri-ictal headache accompanied at least half of their seizures. In over two thirds, peri-ictal headaches presented with symptoms similar to tension-type headache; one quarter reported symptoms that resembled migraine. Nearly half reported severe peri-ictal headaches (visual analog scale >7). Over 70% of the participants with interictal headache used over-the counter analgesics but this was the case in only 40% of those with peri-ictal headache. Of the people who used analgesics for peri-ictal headache, only 10% had sought medical advice for their headache. Peri-ictal headache was seen significantly more in people on anti-epileptic polytherapy, with an earlier age of onset and with generalised tonic-clonic seizures. In comparison, it was seen significantly less in people with absence seizures and simple partial seizures. An advice derived from this study is that each person with epilepsy should be asked explicitly if (s)he also suffer from peri-ictal headache, in order to improve the management of this complaint (Duchaczek *et al.*, 2013). Despite being a common phenomenon that significantly adds to the burden of epilepsy, peri-ictal headache is often underdiagnosed. Studies like these are important to raise awareness amongst physicians about problems associated with seizures. Epileptic seizures themselves have a big impact on people, and this may be the reason why so few people with epilepsy actively mention (severe) peri-ictal headache when they consult their treating physician. People with epilepsy can benefit from an active role of their physician in enquiring about peri-ictal headache so that adequate analgesic treatment can be initiated.

2.1.6 Genetic overlap between epilepsy and migraine

Several genes have been associated with both migraine and epilepsy. Mutations in the three familial hemiplegic migraine (FHM) genes [CACNA1A (FHM1), ATPA1A (FHM2) and SCN1A (FHM3)] can also cause epilepsy (see for review (Haan *et al.*, 2008)). FHM is a very rare monogenetic form of migraine that only accounts for a small number of people with migraine. More important is the search for genetic factors involved in

'normal' migraine and epilepsy, which occur more frequently. Recent genome wide association studies in migraine and epilepsy have given important results, but they can only explain a small genetic contribution to these syndromes. These studies were not aimed at elucidating the co-occurrence of migraine and epilepsy (Anttila *et al.*, 2010; Freilinger *et al.*, 2012; Tan and Berkovic, 2010; de Vries *et al.*, 2009).

One such study reports on a SCNA1A mutation (c3521C>G, p.T1174 S) that was found in a young child with Dravet syndrome (Frosk *et al.*, 2012). SCN1A is a well-known epilepsy gene, that is also associated with FHM3, encodes voltage-gated sodium channels. The child's mother carried this gene mutation and suffered from frequent migraines with aura. The grandmother also suffered from migraines with aura, but genetic analysis could not be performed for her.

The CACNA1A (FHM1) gene causes, among other phenotypes, hemiplegic migraine, epilepsy and episodic ataxia (Haan *et al.*, 2008). A recent analysis of a young woman with paroxysmal sensoriphobia, nausea, vomiting and mild ataxia, but no headache, revealed a novel CACNA1A c3995 +1G>A mutation, leading to a frameshift and premature stop codon (Magis *et al.*, 2012).

Recently, mutations in the proline-rich transmembrane protein (PRRT2) gene have been associated with paroxysmal kinesigenic dyskinesia, benign familial infantile seizures and the infantile convulsion-choreoathetosis syndrome. Mutations in this gene are also hypothesised to be involved in hemiplegic and 'normal' migraine (Gardiner *et al.*, 2012; Marini *et al.*, 2012; Méneret *et al.*, 2013). The proof for the involvement of this gene in migraine is not yet unambiguous, as most studies that found an association with hemiplegic migraine did not exclude the presence of FHM mutations in those patients. The association of mutations in this gene with 'normal' migraine could also be explained by chance due to the high prevalence of migraine in the general population.

The members of a Finnish family with 60 members in which both migraine and epilepsy occurred were interviewed and underwent genetic testing (Polvi *et al.*, 2012). Family members had febrile seizures (12%), epilepsy (22%), migraine (33% without aura, 22% with aura) or attacks of sudden somnolence leading to transient unconsciousness and inability to be awoken (17%). Of family members with migraine, 27% also had epilepsy and of those with epilepsy, 69% also had migraine. Shared loci

for migraine and epilepsy were found on chromosomes 12q24.2-12q24.3 and 14.q12-q23. The first was significantly linked to migraine alone and to the comorbid (epilepsy plus migraine) phenotype. The second was significantly associated with epilepsy with generalised tonic-clonic seizures and migraine (Polvi *et al.*, 2012).

Genetic linkage of migraine and epilepsy has so far only been demonstrated in specific syndromes. Non-syndromal migraine and epilepsy are probably the result of a complex interplay between multiple genes and environmental factors and influences. The combination of genetic analysis with other techniques, such as Transcranial Magnetic Stimulation (TMS, discussed in the next section) could play a major role in elucidating the combined net effect of these factors in the pathogenesis of both conditions.

2.1.7 Cortical excitability in epilepsy and migraine

Dysfunction of ion-channels and associated proteins, caused by genetic mutations, can cause changes in neuronal ion concentration, which in turn leads to changes in cortical excitability (Somjen, 2002). Imbalance between inhibitory and excitatory factors is hypothesised to play a central role in both epilepsy and migraine (Badawy *et al.*, 2009; Coppola and Schoenen, 2012). The aforementioned CACNA1A gene, for example, encodes the alpha subunit of the neuronal voltage-gate calcium channel 2.1. Mutations in this gene were recently shown to alter the affinity of the associated inhibitory G-protein, which potentially reduced inhibition, and caused neurons to become hyperexcitable (Garza-López *et al.*, 2012).

Excitability of the human motor cortex can be tested *in vivo* non-invasively with TMS. Magnetic stimulation of the hand motor cortex is directly monitored with electromyography (EMG) of the corresponding abductor pollicis brevis muscle. Increased cortical excitability is reflected by greater motor evoked potential amplitude and lower motor threshold. With a so-called paired-pulse paradigm, intracortical facilitation (likely glutaminergic) and short and long intracortical inhibition (likely mediated by GABA-A, respectively GABA-B) can be measured. TMS will be discussed in more detail in **chapter 2.2**. Several studies, but not all, have demonstrated cortical hyperexcitability in epilepsy (a complete overview of cortical excitability in epilepsy is provided in **chapter 2.2**), but also in migraine (Aurora *et al.*, 1998, 2003, 2005; Battelli *et al.*, 2002; Brighina *et al.*, 2009; Brigo, Storti, Nardone, *et al.*, 2012; Conforto *et al.*, 2012; Gerwig *et al.*, 2012; Mulleners *et al.*, 2001; Ozturk *et al.*, 2002; Siniatchkin *et al.*, 2009; Werhahn, Wiseman, *et al.*, 2000) and FHM (van der Kamp *et al.*, 1997; Werhahn,

Wiseman, *et al.*, 2000). In migraine, cortical hyperexcitability is found predominantly in the occipital cortex, while motor excitability is mostly normal. In epilepsy, excitability measures derived from the motor cortex are elevated in most studies (see **chapter 2.2**). Recently, the first TMS study to directly compare cortical excitability between subjects with epilepsy, migraine and healthy controls was published (Badawy and Jackson, 2012) Drug-naive people with migraine (N=25) or epilepsy (N=50) were included in this study. The cortex of people with migraine or epilepsy was hyperexcitable compared to the cortex of healthy controls. The motor threshold was not significantly different; however, recovery curves were abnormal in both groups. In healthy controls, (GABA-B mediated) inhibition at an interstimulus interval of 150 and 250ms was apparent, reflected by a conditioning response larger than the test response. In subjects with migraine or epilepsy this inhibition was lacking, probably indicating impaired GABA-B inhibition. This was more pronounced in subjects with epilepsy, but still significant in subjects suffering from migraine. These results are considered as first *in vivo* evidence of impaired GABA-B mediated inhibition in both conditions in humans (Badawy and Jackson, 2012). GABA-B receptors are also G-protein coupled receptors that are also associated to calcium channels. Speculatively, in epilepsy and migraine, calcium channel mutations and other ionchannel mutations may impair GABA-B receptor function, thereby causing hyperexcitability. Other studies have also demonstrated impaired GABA-B -ergic inhibition, or low GABA-B receptor expression in (animal models of) epilepsy (Brown *et al.*, 2003; Lavallee *et al.*, 2011; Merlo *et al.*, 2007) and migraine (Holland *et al.*, 2010; Plummer *et al.*, 2011). One study found an association between migraine and mutations in the GABA-A receptor-coding locus in humans (Russo *et al.*, 2005) but this was not replicated in later studies (Fernandez *et al.*, 2008; Netzer *et al.*, 2008; Oswell *et al.*, 2008). GABA-A mutations have, however, consistently been found in various forms of epilepsy (Baulac *et al.*, 2001; Lachance-Touchette *et al.*, 2011; Wallace *et al.*, 2001).

There is a growing body of evidence that (GABA-mediated) cortical inhibition is reduced in migraine as well as in epilepsy, but important questions remain. Are migraine and epilepsy different entities of the same continuum? This seems to be in contradiction with the low occurrence of migralepsy, so it is possible that the multifactorial pathways that result in cortical hyperexcitability are different in both conditions, and lead to different types of paroxysmal symptoms (seizures and migraine headache). In the next section, I review studies that attempt to answer these questions.

2.1.8 Cortical spreading depression and epileptogenicity

Spreading depression is thought to play a key role in migraine pathophysiology, as well as in other neurological conditions such as stroke, subarachnoid haemorrhage and traumatic brain injury (Dreier, 2011; Lauritzen *et al.*, 2011). Cortical spreading depression (CSD) is probably the epiphenomenon of spreading depolarisation caused by a depolarisation block of neuronal activity, although other mechanisms may also be involved (Dreier, 2011). In animal models, spreading depression was preceded by a small band of fast-oscillating activity, presumably indicating initial increased cortical excitability (Herreras *et al.*, 1994; Larrosa *et al.*, 2006). This, in addition to epidemiological and genetic links between migraine and epilepsy discussed above, suggests that both conditions have a common pathophysiological mechanism. A computational study of neuronal membrane dynamics suggested that seizures, spreading depression and normal spiking behaviour of neurons are all part of the same dynamic physiological continuum and that transitions between these different states is determined by a small set of parameters such as oxygen and potassium (Wei *et al.*, 2014). This may explain why, in certain circumstances, both spreading depression and epileptic features have been found in human intracranial EEG recordings.

The first EEG correlates of spreading depression were measured in humans after the development of intracranial EEG electrodes as the dura mater and the skull filter most of the slow voltage changes that are associated with spreading depression, (Mayevsky *et al.*, 1996). Several reports of spreading depression in humans, mostly with traumatic brain injury or subarachnoid haemorrhage were published since (Fabricius *et al.*, 2006; Strong *et al.*, 2002). Harreveld and Stamm first described spreading convulsions that were elicited after repeated induction of cortical stimulation. Spreading convulsions were defined as a spreading depolarisation with ictal epileptic field potentials riding on the final shoulder of the slow potential change where, normally, depression of spontaneous activity is observed (cited in (Dreier *et al.*, 2012)).

The relation between CSD and epileptic activity was further investigated in subjects with subarachnoid haemorrhage using intracranial recordings that were implanted for monitoring purposes (Dreier *et al.*, 2012). This study is the first to report the recording of spreading convulsions in humans. Of the twenty-five subjects included, two showed spreading convulsions. Three people had 55 isolated ictal epileptic events. In 21 people, a total of 656 spreading depressions were recorded. The depression period per day and

number of spreading depolarisations per day peaked on the day of the subarachnoid haemorrhage and on day 7 after the event. The number of ictal events peaked on day 8. Of the seven people that could be assessed clinically (Glasgow coma score >13), six had spreading depression. Interestingly, these people did not report symptoms similar to migraine aura. Two of these people deteriorated on the seventh day to Glasgow coma score 3. Eighteen people were available for follow-up. Of these, eight (44%) had developed seizures within 3 years of the subarachnoid haemorrhage. They had a higher peak number of spreading depolarisations and the peak depression period tended to be longer than in people without seizures. In people with poor outcome (Glasgow coma score 1-4), the depression period per recording day was significantly longer than in people with good outcome. The two people who had spreading convulsions were later readmitted to the hospital with status epilepticus (Dreier *et al.*, 2012).

To test the role of GABA inhibition in spreading convulsions, an *in vitro* experiment was conducted (Dreier *et al.*, 2012). Potassium chloride was injected into neocortical slices that were surgically resected from people with epilepsy, to trigger a spreading depolarisation. In three slices from different people ictal epileptic field potentials were triggered. After addition of the GABA antagonist bicuculline, the spreading depolarisation triggered ictal epileptic field potentials in more slices. This effect was reversed in 14 of 16 slices after bicuculline was washed out. The authors conclude that impairment of GABA inhibition causes epileptic field potentials seen in spreading convulsions (Dreier *et al.*, 2012). This study also demonstrates the association between early spreading depolarisation and epileptic activity and outcome and underscores the complexity of the interplay between epilepsy and spreading depolarisation.

The threshold for cortical spreading depression was increased in a model of acute symptomatic epilepsy (Tomkins *et al.*, 2007). In humans, cortical spreading depression can co-occur with epileptic activity in acute brain injury (Fabricius *et al.*, 2009). Repeated cortical spreading depression appears to increase epileptic activity *in vitro*, due to suppression of inhibitory GABA function (Gorji and Speckmann, 2004; Krüger *et al.*, 1996). It is possible that an intrinsic protection mechanism against cortical spreading depression exists in chronic epilepsy, as was suggested by the strongly increased threshold for cortical spreading depression in brain slices from subjects suffering from chronic epilepsy (Dreier *et al.*, 2012). This finding is in agreement with the results of an earlier *in vitro* study, in which the threshold for cortical spreading

depression in neocortical slices from human subjects and rats with chronic refractory epilepsy were compared to the cortical spreading depression threshold of age-matched and younger rats without epilepsy (Maslarova *et al.*, 2011). The slices of human subjects and rats with epilepsy had higher thresholds for cortical spreading depression than the slices of young and old healthy rats. Application of a GABA antagonist lowered the threshold similarly in all types of tissue, leading the authors to conclude that the higher threshold for cortical spreading depression in epilepsy is probably not due to altered GABA-ergic function (Maslarova *et al.*, 2011). Dreier *et* al. speculate that migraine aura would occur more frequently in chronic epilepsy if this "intrinsic protective mechanism" of higher threshold did not exist (Dreier *et al.*, 2012).

Studies in FHM knock-in mice with a R192Q and S218L mutation revealed increased neuronal calcium influx and neurotransmitter release, and an increased susceptibility to cortical spreading depression upon topical cortical application of potassium chloride or current injection (van den Maagdenberg *et al.*, 2004, 2010). This experimental cortical spreading depression also caused a temporary hemiparesis in mutant mice, but no obvious epileptic phenomena. In the same study it was shown that inhibitory neurotransmission seemed unaffected by the mutations. The predicted functional consequence is that FHM mutations lead to increased levels of glutamate and potassium in the synaptic cleft. This results in an increased propensity for cortical spreading depression, since both are considered facilitators of this phenomenon.

Cortical hyperexcitability seems to underlie both migraine and epilepsy. What pathophysiologically stands both conditions apart is the fast, synchronous neuronal activity that is seen in epilepsy but not in migraine. The challenge for future studies is to further develop understanding of the underlying cause for this fast activity and of the factors that initiate a seizure or migraine attack. In **chapters 6 and 8** I investigate how the brain response to TMS differs in people with epilepsy or migraine and people without these conditions. I show how this helps to understand the different and common processes underlying epilepsy and migraine.

2.1.9 Summary and conclusion

Several recent developments have shed more light on the complex relationship between epilepsy and headache. Results of epidemiologic studies on headache and epilepsy in adults remain conflicting, but the association of migraine with epilepsy seems robust, especially in paediatric populations. An explanation for this observation

could be that childhood epilepsy is more likely to be caused by genetic factors affecting ion channel and neurotransmitter function than (acquired) epilepsy that occurs later in life. The clear association between epilepsies with a genetic cause, such as JME and benign epilepsy with centrotemporal spikes, and migraine supports this explanation.

The present classification of conditions in which headache and epilepsy co-occur in time (migralepsy, hemicrania epileptica, postictal headache) is challenging, as EEG recordings are not routinely done in case of headache without other neurological symptoms. So far, it appears impossible to differentiate so-called 'ictal epileptic headache' from other types of headache purely based on symptomatology. Ictal epileptic headache will probably remain underdiagnosed, but an ictal EEG recording can help diagnosis when confronted to a person with recurring severe headaches that are difficult to treat. Peri-ictal headache is, despite the relatively easy history-based diagnosis, underdiagnosed and therefore undertreated. This presents a large burden in people with epilepsy and an active effort of physicians is needed to improve diagnosis and treatment (Duchaczek *et al.*, 2013).

Evidence increasingly points to a link between epilepsy and migraine that probably involves functional alterations of membrane channels and neurotransmitters influencing cortical excitability (Badawy and Jackson, 2012; Haan *et al.*, 2008). Imbalance between excitatory (glutamate) and inhibitory (GABA) factors, especially GABA-B receptor dysfunction, seems to play a pivotal role in epilepsy, and possibly also in migraine. Future efforts should be directed at further understanding the role of these factors in migraine.

Genetic epilepsies are often caused by gene mutations that influence cortical excitability and may thus alter susceptibility to spreading depression and migraine. *In vitro* studies demonstrated a higher threshold for spreading depression in surgically resected brain slices from subjects with longstanding localisation related epilepsy (Dreier *et al.*, 2012; Maslarova *et al.*, 2011). This would explain why epilepsies with a likely genetic cause such as rolandic epilepsy and JME may be more often co-morbid with migraine (Kelley *et al.*, 2012; Schankin *et al.*, 2011; Winawer and Connors, 2013).

The need for invasive EEG monitoring in acute, severe brain injury also offers a valuable opportunity to improve understanding of cortical spreading depression in

humans. Cortical spreading depression and epileptiform activity may influence each other in a reciprocal manner in acute brain injury (Dreier *et al.*, 2012). Seizures are seen in acute brain injury, but reports of migraine aura are remarkably scarce (Dreier *et al.*, 2001). It can be expected, based on these results that subclinical spreading depolarisations occur more often than epileptic events in acute brain injury (Dreier *et al.*, 2012). It is currently impossible to test this hypothesis as invasive EEG monitoring is only used in the most severe cases of brain injury. Studies involving other, non-invasive, techniques are warranted to study this complex relationship.

2.2 Measuring cortical excitability in epilepsy

2.2.1 Transcranial Magnetic Stimulation and cortical excitability

The term "cortical excitability" was briefly introduced in the past sections. But what exactly is cortical excitability? Why is it so important? And how can it be measured? In the following section I attempt to answer these questions by providing an overview of the literature on this topic. First, I describe the factors that influence cortical excitability. Then I summarise findings of previous studies that have measured cortical excitability in people with epilepsy using Transcranial Magnetic Stimulation (TMS) and discuss the potential role of this technique in the clinical evaluation of epilepsy, treatment monitoring, and outcome prediction. Studies investigating the therapeutic use of TMS, such as through repetitive stimulation are beyond the scope of this thesis.

Neuronal excitability can be defined as the readiness of a neuron to generate an action potential when triggered, usually by an excitatory post-synaptic potential (Burke *et al.*, 2001; Debanne *et al.*, 2003). Cortical excitability depends on many factors, amongst others the membrane potential, which directly influences how close neurons are to firing threshold. The membrane potential of neurons in the brain is tightly regulated by ion channels. Even when sudden changes in ion concentration occur in the body, ion concentration in the brain is unaffected (Burke *et al.*, 2001; Somjen, 2002). The main chemicals involved in this process are K^+, Na^+, Ca^{2+}, H^+, Mg^+, Cl^- and HCO^{3-}. Ion channels and neurotransmitters are functionally interconnected. Some ion channels are neurotransmitter-gated, and Ca^{2+} influx into a neuron causes the release of neurotransmitters into the synaptic cleft, in turn influencing other neurons and their ion channels. Cortical excitability is also determined by neurotransmitters such as GABA, which has an inhibitory effect, and glutamate, which has an excitatory effect

(Badawy, Harvey, *et al.*, 2009; Somjen, 2002). There is increasing evidence that interneurons and the connectivity between different brain regions, neurons and interneurons are important determinants of cortical excitability (Giambattistelli *et al.*, 2014; Wendling *et al.*, 2016)

Cortical excitability can be measured *in vivo* using transcranial electrical stimulation and TMS combined with electromyography (EMG) and/or EEG (see figure 2.1A) (Abbruzzese and Trompetto, 2002). Since first described in 1984 (see Barker *et al.*, 1985), TMS has developed into a valuable tool for neuroscientific research (Kobayashi and Pascual-Leone, 2003). TMS has some advantages over transcranial electrical stimulation as participants do not need to be sedated and it is less uncomfortable then electrical stimulation. TMS, like transcranial electrical stimulation, has excellent temporal resolution. Spatial resolution is around 1 cm but as TMS only reaches 2 cm from the skull only superficial brain areas can be investigated.

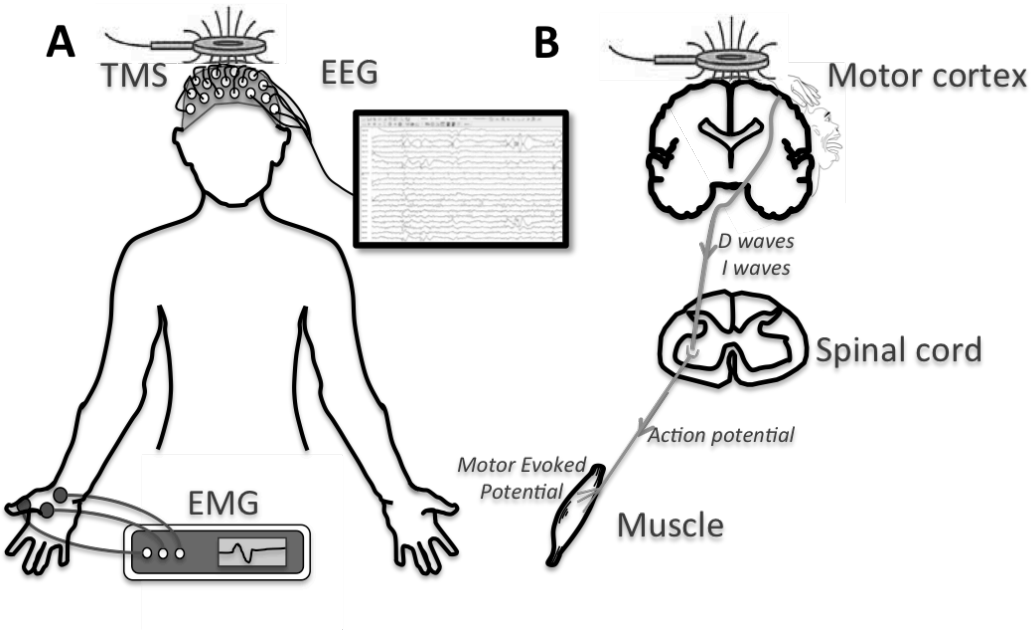

Figure 2.1: Transcranial magnetic stimulation set-up and physiology. *A: set-up for transcranial magnetic stimulation (TMS) with electromyography (EMG) and electroencephalography (EEG). B: schematic representation of the physiology underlying the motor evoked potential (MEP). A TMS pulse triggers descending volleys of action potentials from the motor cortex to the spinal cord, causing glutamate release in the cortico-motor neuronal synapses. If they exceed the firing threshold, an action potential is triggered in the spinal motor neurons, which propagates along the peripheral motor axons and induces a motor response (MEP) in the muscle.*

A TMS pulse activates all neurons within reach, without differentiating between inhibitory, excitatory, or modulating neurons. The resulting effect is always a non-specific sum of the effects of the activated neuron population, comprising both neurons and interneurons (Huerta and Volpe, 2009). A TMS pulse depolarises neurons by inducing electric fields in the tissue, which causes neurons to fire and the pulse to spread to neighbouring neurons. Stimulation of the motor cortex triggers descending volleys of action potentials to the spinal cord that cause glutamate release in the cortico-motor neuronal synapses. If they exceed the firing threshold, an action potential is triggered in the spinal motor neurons, which propagates along the peripheral motor axons and induces a motor response (motor evoked potential, MEP, see figure 2.1 B)(Groppa *et al.*, 2012). Descending volleys can be measured using epidural recordings in the spinal cord. There are two types of volleys: direct (D), originating from direct stimulation of corticospinal neurons, or indirect (I), originating from synaptic activation of corticospinal neurons (Kobayashi and Pascual-Leone, 2003). In the hand area, TMS stimulation perpendicular to the central sulcus mostly generates I-waves. D-waves are found at higher stimulus intensity (Abbruzzese and Trompetto, 2002). This is probably due to the orientation of the neuronal population activated. The interneurons in the hand motor area are orientated parallel to the skull surface, making them more sensitive to magnetic field impulses. In contrast, latero-medial stimulation generates more D-waves (Abbruzzese and Trompetto, 2002). The effect of a TMS pulse on the cortex, reflecting cortical excitability, can be measured in several ways. The most commonly used method is to measure a MEP using surface EMG recordings of the muscle group that is stimulated in the brain. Both D- and I-waves contribute to the EMG response, and excitability of both spinal and cortical motor neurons influence the MEP (Abbruzzese and Trompetto, 2002). Another novel but technically more challenging possibility is to combine TMS with EEG, enabling the measurement of cortical excitability in areas outside the motor cortex (Miniussi and Thut, 2010). I will discuss this technique in **section 2.2.4**, and describe my own TMS-EEG study in **chapters 6 and 8**.

Several variables of the MEP can be measured (see table 2.2). The MEP amplitude is influenced by the number of motor neurons recruited in the spinal chord, and the synchronisation of discharges of the motor neurons upon the TMS pulse. Cortical stimulation causes repetitive, but asynchronous, discharges of spinal motor neurons (Z'Graggen et al., 2005). The MEP amplitude is highly variable - from pulse to pulse,

and between individuals. The main causes of the high variability are desynchronisation and phase cancellation of the action potentials within the corticospinal tract (Magistris *et al.*, 1998; Z'Graggen *et al.*, 2005). The MEP amplitude is roughly proportional to the stimulus intensity and saturates at high stimulus intensities. Due to the variability, the MEP amplitude is not often used as a clinical marker of cortical excitability. The resting motor threshold (rMT) is the lowest TMS pulse intensity that triggers reproducible MEPs (typically of >50uV in a fully relaxed target muscle) in 50% of trials (Groppa et al., 2012). This was proven to be a reliable and repeatable measure of cortical excitability (Badawy, Jackson, et al., 2012).

The active motor threshold (aMT) represents the motor threshold of a slightly contracted muscle and is lower than the rMT. It is defined as the required stimulus intensity to elicit reproducible MEPs of 200-300uV in 50% of consecutive trials (Wassermann *et al.*, 2008). The amplitude of MEPs at the aMT is higher than that at the rMT, due to spinal facilitation. The rMT also decreases when a subject imagines contracting the target muscle. This facilitation is thought to originate entirely from cortical mechanisms (Abbruzzese and Trompetto, 2002).

Table 2.2: Key TMS measures in the context of cortical excitability.

TMS measure	Mechanism	Increased excitability	Decreased excitability
Motor evoked potential (MEP)	membrane potential	increased	decreased
Motor threshold (MT)	membrane potential	lower	higher
Cortical silent period (cSP)	GABA-B receptor	shorter	longer
Short intracortical inhibition (SICI)	GABA-A receptor	lower	higher
Long intracortical inhibition (LICI)	GABA-B receptor	lower	higher
Intracortical facilitation (ICF)	NMDA,Glutamate receptor	higher	lower

After a single TMS pulse, a MEP is followed by a silent period (SP) lasting up to 300ms, during which there is no EMG signal. The length of the SP is directly related to the stimulus intensity (Terao and Ugawa, 2002). The mechanism underlying the SP is complex. During the first 50ms post-stimulus, the amplitude of the peripheral Hoffmann reflex also decreases or disappears, pointing towards a spinal origin for this first part of the silent period (Fuhr *et al.*, 1991). Paired-pulse stimulation has shed more light on the later part of the silent period. When two suprathreshold stimuli are

applied with an interstimulus interval of 50 to 150ms, MEPs remained constant. A subthreshold stimulus following 50-150ms after a suprathreshold stimulus however, showed inhibition of the response (Roick *et al.*, 1993). Subthreshold stimuli activate neurons indirectly, probably through trans-synaptic mechanisms, whereas suprathreshold stimulation can directly activate corticospinal neurons (Edgley *et al.*, 1990). Thus, it is probable that the later part of the silent period originates from trans-synaptic activation in the cortex. It is therefore termed the cortical silent period (cSP). As the duration of the cSP coincides with the timing of GABA-B receptor activation, the late part of the cSP is believed to be mediated by GABA-B (Connors *et al.*, 1988). Pharmacological evidence is somewhat conflicting: the selective GABA-B agonist baclofen was shown in some studies to increase the cSP (Siebner *et al.*, 1998; Stetkarova and Kofler, 2013), whereas other studies did not show any effect (Inghilleri *et al.*, 1996; McDonnell *et al.*, 2006). This may have been due to the way the drug was administered, as the two studies that showed a lengthening of the cSP administered baclofen intrathecally, whereas the other studies used oral or intravenous administration. GABA-A agonists such as lorazepam and ethanol have also been shown to increase the cSP length (Ziemann, 2004). At higher stimulus intensities, however, lorazepam reduced cSP length, possibly reflecting an interaction between GABA-A- and GABA-B- receptors (Kimiskidis *et al.*, 2006). Tiagabine, a GABA re-uptake inhibitor, lengthens the cSP (Werhahn *et al.*, 1999).

Paired-pulse TMS protocols are widely used to study excitability of the motor cortex and its underlying mechanisms (Kujirai *et al.*, 1993). To this end, two sub- or supra-threshold pulses are applied, with an interstimulus interval of between two and 400ms. Short-interval intracortical inhibition (SICI) is studied with a first stimulus at subthreshold intensity and a second, suprathreshold, stimulus 1-6ms after the first stimulus. SICI has two distinct phases (Fisher *et al.*, 2002; Hanajima *et al.*, 2003). At an interstimulus interval of 1ms both I- and D- waves are suppressed, which may be caused by axonal refractoriness (Hanajima *et al.*, 2003). At an interstimulus interval of 3-5ms, I-waves (especially the later I3-waves) are selectively inhibited (Hanajima *et al.*, 2003). This inhibition lasts 20ms and is thought to be mediated by GABA-A (Hanajima *et al.*, 2003; Kujirai *et al.*, 1993). This notion was supported by pharmaceutical evidence as GABA-A agonists such as diazepam, lorazepam and ethanol increase SICI (Paulus *et al.*, 2008).

Intracortical facilitation (ICF) occurs when the test stimulus follows the conditioning stimulus after 10-15ms (Kujirai *et al.*, 1993). It has been assessed less extensively than SICI and its exact mechanism of action remains unknown. The Hoffmann reflex is not facilitated by the conditioning stimulus, making a cortical origin of this mechanism likely (Ziemann *et al.*, 1996). It has, however, never been demonstrated that the I-waves are increased in number or amplitude at interstimulus intervals of 10-15ms, so that a spinal mechanism may be involved in ICF. This is thought to be unlikely (Di Lazzaro *et al.*, 2006). NMDA-receptor antagonists such as dextromethorphan reduce ICF (Ziemann, Chen, *et al.*, 1998) as well as GABA-A receptor agonists (Paulus *et al.*, 2008). The glutamate antagonist riluzole suppresses ICF (Schwenkreis *et al.*, 2000).

With two suprathreshold pulses, long-interval intracortical inhibition (LICI) can be demonstrated at interstimulus intervals of 50-200ms. This is likely to be a phenomenon of cortical origin as the I-waves, but not the D-waves, are affected (Nakamura *et al.*, 1997). Baclofen increases LICI (McDonnell *et al.*, 2006). These findings, taken together, suggest that LICI is probably GABA-B mediated (Nakamura *et al.*, 1997; Wassermann *et al.*, 2008). GABA-A receptors are ligand-gated ion channels and act faster than the G-protein coupled GABA-B receptors, explaining part of the different timing between SICI and LICI (Nakamura *et al.*, 1997).

Measuring the change in MEP response, after various combinations of conditioning and test stimuli, is limited by the inter-individual variability of the MEP response in each set of conditioning/test stimuli, and the ensuing requirement for several consecutive measurements (Cahn *et al.*, 2003). An alternative is to target a constant MEP amplitude output, set as a percentage of the maximum response of a baseline test, and to track this by changing the test stimulus intensity. Changes in cortical excitability are then reflected by the required change in test stimulus intensity to result in the preset output (Awiszus *et al.*, 1999; Bostock *et al.*, 1998; Vucic *et al.*, 2006). With this threshold tracking technique (or "threshold hunting"), there is tighter control of the necessary pulse and small differences in cortical excitability can be measured more easily. Moreover, spinal and peripheral influences on measurements are reduced. It was shown that this method is a valid alternative to constant stimulus

methods (Vucic *et al.*, 2006). Recently, this method has suggested different mechanisms influencing cortical excitability (Fisher *et al.*, 2002; Vucic *et al.*, 2011).

Cortical excitability in behaviourally silent areas can be measured by combining TMS with electroencephalography (EEG) (Ilmoniemi *et al.*, 1997; Izumi *et al.*, 1997; Izurnia *et al.*, 1997). This is technically challenging as TMS pulses can lead to large artefacts on EEG recordings (Ilmoniemi and Kicić, 2010; Ilmoniemi *et al.*, 1997; Veniero *et al.*, 2009), but the combination of these techniques is valuable in uncovering neural mechanisms, including changes in cortical excitability (Daskalakis *et al.*, 2012; Komssi and Kähkönen, 2006; Miniussi and Thut, 2010). The EEG response to TMS over the motor cortex in healthy individuals consists of positive peaks at 30, 60 and 150ms after the TMS pulse, and negative peaks at 15, 45 and 100ms (Rogasch and Fitzgerald, 2012). TMS-EEG responses have been shown to be repeatable and stable over time (Casarotto *et al.*, 2010; Lioumis *et al.*, 2009).

Single- and paired- pulse TMS protocols are considered safe as a diagnostic tool (Rossi *et al.*, 2009). Some side effects of TMS were reported, such as seizures, which mostly occurred in people with underlying brain pathology or those taking neuroactive medication (Groppa *et al.*, 2012; Hömberg and Netz, 1989; Rossi *et al.*, 2009). Syncope was also reported during TMS, although is not thought to be a direct effect of TMS, but rather related to anxiety and stress during the procedure (Groppa *et al.*, 2012; Hadar *et al.*, 2011). Temporary hearing loss may occur due to the coil click if no hearing protection is used, and stimulation can cause short-term headaches, local pain and paresthesias (Rossi *et al.*, 2009). No histologic changes in brain tissue (temporal lobes) were found in post-mortem examinations in people who underwent TMS and died from an unrelated cause (Gates *et al.*, 1992).

In people with epilepsy, no adverse effects of single-pulse TMS have been observed in the vast majority of studies (Tassinari *et al.*, 2003; Ziemann, Steinhoff, *et al.*, 1998). The risk of TMS-associated seizures in people with epilepsy is unclear, as adverse effects of TMS are not always reported. Forty-nine articles including a total of 712 people with epilepsy who underwent single- and paired- pulse TMS have been systematically reviewed (Schrader *et al.*, 2004). Only 22 studies (with a total of 458 subjects) reported adverse effects of TMS. In these 22 studies, seven subjects had a seizure (1.5%), and five

of these were from the same clinic (Hufnagel and Elger, 1991). Group analysis suggested that the crude risk of seizure occurrence during single- and paired- pulse TMS was lowest (0%) in people with well-controlled epilepsy (Schrader *et al.*, 2004). The risk of seizure occurrence was highest (2.8%) if AEDs were tapered. No explanation was found for the described centre-to-centre variability. There is no clear pathophysiological evidence suggesting that TMS triggers seizures in people with epilepsy, instead the reported seizures during TMS may have been coincidental in people who have frequent seizures. A study assessing seizure risk in which individual seizure frequency is taken into account is necessary to address this question.

2.2.2 Influences on cortical excitability

Cortical excitability is dynamic and varies depending on physiological as well as external conditions. When using TMS it is of paramount importance to take these potential confounders into account. Several studies evaluating the effects on TMS parameters in healthy individuals are listed in table 2.3. The most important influences are discussed below.

Circadian rhythm

Muscle power increases throughout the day, but MEP latency, the cSP (Strutton *et al.*, 2003), ICF, and SICI (Doeltgen and Ridding, 2010) stay relatively constant. Using TMS-EEG, the TMS Evoked Potential (TEP) slope and amplitude have been shown to increase throughout the day (Huber *et al.*, 2013). LICI and cSP decreased over the course of a day (Barker *et al.*, 1985). Sleep deprivation seems to increase cortical excitability. It has been shown to reduce intracortical inhibition (Civardi *et al.*, 2001; Kreuzer *et al.*, 2011) and to increase TEP slope and amplitude (Huber *et al.*, 2013). Sleep deprivation increased ICF only in women, raising questions about the underlying mechanisms (De Gennaro *et al.*, 2007).

Hormones

Cortical excitability varies throughout the menstrual cycle. Oestrogens have an excitatory effect while progesterone has an inhibitory effect (Finocchi and Ferrari, 2011; Smith *et al.*, 2002). In the early follicular phase, when both hormone levels are relatively low, the tendency is towards inhibition. In the late follicular phase, with high

oestradiol and low progesterone levels, excitability rises. In the luteal phase, progesterone levels are higher than oestradiol levels and excitability returns to around the same levels as during the early follicular phase (Smith *et al.*, 2002). In women with anovulatory cycles, inhibition is increased during menstruation, possibly reflecting the withdrawal of estrogens and their excitatory effect (Hattemer *et al.*, 2007). One study did not find a significantly different rMT in healthy subjects and women suffering from migraine during their menstrual cycle. In the same study, no significant difference in cortical excitability was seen between women taking oral contraceptives and those who did not (Boros *et al.*, 2009).

Medication

Many pharmacological substances that act on the central nervous system were studied with TMS, and good overviews of these studies are available (Paulus *et al.*, 2008; Ziemann, 2004). I will only discuss the effect on TMS parameters of the most important AEDs, without aiming to provide an exhaustive list. Na+ blockers such as lamotrigine, carbamazepine and phenytoin increase the motor threshold, but do not affect other TMS parameters (Chen *et al.*, 1997; Lang *et al.*, 2013; Li *et al.*, 2009; Turazzini *et al.*, 2004). Benzodiazepines such as diazepam and lorazepam are GABA-A receptor agonists. They have no effect on the MT but increase SICI (Inghilleri *et al.*, 1996; Kimiskidis *et al.*, 2006; Di Lazzaro *et al.*, 2005). Diazepam decreases the cSP whereas lorazepam increases the cSP at high stimulus intensities but decreases the cSP at low stimulus intensities (Inghilleri *et al.*, 1996; Kimiskidis *et al.*, 2006; Ziemann, 2004). Tiagabine, a GABA re-uptake inhibitor, and vigabatrin, an inhibitor of the GABA transaminase that breaks down GABA, both enhance GABA function, resulting in a prolonged cSP and stronger LICI (Pierantozzi *et al.*, 2004; Werhahn *et al.*, 1999). Valproic acid acts on sodium and calcium channels, and also inhibits GABA transaminase, enhancing GABA-ergic inhibition. Only one TMS study is available on this drug. It showed an increase of the MT and no effect on the cSP (Li *et al.*, 2009). The working mechanism of topiramate is not well known. It increases SICI but has no measurable effect on the MT and the cSP (Reis *et al.*, 2002). Similarly, the mechanism of action of levetiracetam is unknown. TMS studies have demonstrated an increased MT and cSP duration upon levetiracetam administration, but no effect on SICI and ICF (Reis *et al.*, 2004; Solinas *et al.*, 2008). Anti-epileptic drugs thus reduce cortical excitability through various mechanisms.

Neuroactive substances

Coffee is the most widely used neurostimulative substance. Two studies demonstrated a reduction of the cSP after administration of caffeine, but no influence on other cortical excitability measures (de Carvalho *et al.*, 2010; Cerqueira *et al.*, 2006). An earlier study showed no significant effect of caffeine on the resting or active MT, SICI, or ICF (Orth *et al.*, 2005). Another study showed an increase in MEP size after the consumption of an energy drink containing water, sugar and caffeine, an effect which was not seen when subjects were given water alone (Specterman *et al.*, 2005). Spinal excitability was also increased by caffeine (Walton *et al.*, 2003).

Alcohol decreases cortical excitability, especially in the right prefrontal areas (Kahkönen, 2005; Kähkönen and Wilenius, 2007; Kahkönen *et al.*, 2003; Kähkönen *et al.*, 2001). This is reflected by an increase of the cSP and SICI, while the ICF decreases (Paulus *et al.*, 2008). The effect of nicotine and smoking was assessed in one study, which showed that cortical excitability is lower in chronic smokers than in non-smokers (Lang *et al.*, 2008). Few studies have investigated the effects of drug abuse on cortical excitability. In chronic cannabis users, cortical excitability is increased. Users were found to have reduced SICI, irrespective of the frequency of cannabis use around the time of the experiment. Other measures of cortical excitability are not affected by cannabis use (Fitzgerald *et al.*, 2009). Schizophrenia and substance abuse often co-exist. People who had a history of cannabis use when they suffered their first schizophrenic episode had increased cortical excitability. Cannabis users had lower cortical inhibition and higher ICF than people who had no history of cannabis use when they suffered from their first schizophrenic episode (Wobrock *et al.*, 2010). Cocaine increases the active and resting MT, leading to a decrease in cortical excitability. The cSP of cocaine users was not significantly different from healthy controls (Boutros *et al.*, 2005).

Other external influences

As electromagnetic fields can alter cortical excitability, the effect of mobile phone use has been investigated. Mobile phone use was found to increase cortical excitability in the exposed hemisphere, reflected by an enhanced ICF and reduced SICI (Ferreri *et al.*, 2006). These findings were replicated in a group of people with focal epilepsy, but

increase of cortical excitability was only evident in the hemisphere contralateral to the epileptic hemisphere (Tombini *et al.*, 2013). To date, however, there is no evidence for, or reports of, an actual increase in seizures related to mobile-phone usage, even though mobile phones are increasingly used in day-to-day life.

Table 2.3: Influences on variables related to cortical excitability.

Influence	Study	MEP ampl	rMT	aMT	cSP	SICI	LICI	ICF
Circadian rhythm	Doeltgen & Ridding, 2010					↔		↔
	Strutton *et al.*, 2003				↔			
	Lang *et al.*, 2011				↓		↓	
Sleep deprivation	Civardi *et al.*, 2001					↓		
	Kreuzer *et al.*, 2011					↓		
	De Gennaro *et al.*, 2007							↑**
Early follicular	Smith *et al.*, 2002					↑		
	Smith *et al.*, 1999		↔			↑		↑
	Inghilleri *et al.*, 2004	↓						
	Hattemer *et al.*, 2007		↔		↔	↔		↔
Late follicular	Smith *et al.*, 2002					↓		
	Smith *et al.*, 1999							
	Inghilleri *et al.*, 2004	↑						
	Hattemer *et al.*, 2007		↔		↔	↑*		↔
Luteal phase	Smith *et al.*, 2002					↓		
	Smith *et al.*, 1999		↔			↓		↓
	Inghilleri *et al.*, 2004							
	Hattemer *et al.*, 2007		↔		↔	↔		↔
Na+/Ca+ blockers	Paulus *et al.*, 2008		↑	↑				
GABA-B R agonist	Paulus *et al.*, 2008	↓			↑	↑		↓
Coffee	Cerqueira *et al.*, 2006				↓			
	de Carvalho *et al.*, 2010				↓			
	Orth *et al.*, 2005			↔		↔		↔
	Specterman *et al.*, 2005	↑						
Alcohol	Ziemann *et al.*, 1995		↔	↔	↑	↑		↓
Cannabis	Fitzgerald *et al.*, 2009	↔	↔	↔	↔	↑	↔	↔
Cocaine	Boutros *et al.*, 2005		↑ ^	↑	↔			
Ketogenic diet	Cantello *et al.*, 2007		↔		↔	↑		↔
Mobile phone use	Ferreri *et al.*, 2006	↑				↓		↑
Meditation	Guglietti *et al.*, 2012				↑	↔		

** only significant effect during anovulatory phase. No effect in ovulatory phase. **only in female subjects. ^ right hemisphere. MEP amplitude: amplitude of motor evoked potential. rMT: resting motor threshold. aMT: active motor threshold. cSP: cortical silent period. SICI: short-interval intracortical inhibition. LICI: long-interval intracortical inhibition. ICF: intracortical facilitation.*

Recently, the effect of meditation on TMS parameters was studied (Guglietti *et al.*, 2012). There was an increase in the cSP compared to the control group who watched

television, indicating a decrease in excitability. SICI was not altered in the people who meditated.

2.2.3 Epilepsy and cortical excitability

There are many TMS studies in people with epilepsy, which I will review in this section. I conducted a search in PubMed in November 2012, using the search terms "transcranial magnetic stimulation" AND "epilepsy", and identified 381 articles. For the review, I included articles describing original research, written in English, describing a single- and/or paired- pulse TMS protocol conducted in people with epilepsy. After excluding studies using repetitive TMS, and case-reports including fewer than five subjects, 63 articles remained for further reading. By checking the reference lists, I added four more articles. A further seventeen articles were excluded; nine reported on fewer than five subjects, three reported on people without epilepsy, two did not report primary data, one concerned TMS-EEG findings, and for two studies, only the abstract was available. I included a total of 50 articles in the review, shown in tables 2.4, 2.5, and 2.6. It was unclear whether studies by the same authors used overlapping subject groups (see also **chapter 7.4**). The methods of the different centres vary too widely to do a meta-analysis.

Generalised epilepsy

The MT was normal in most studies that included subjects with idiopathic generalised epilepsy (old classification,) without medication (Badawy *et al.*, 2006, 2007; Badawy, Jackson, *et al.*, 2012; Badawy, Macdonell, *et al.*, 2012; Cantello *et al.*, 2006; Joo *et al.*, 2008; Klimpe *et al.*, 2009; Macdonell *et al.*, 2001). Some studies found a decreased MT in people without medication, but increased MT in people with medication (Aguglia *et al.*, 2000; Badawy, Macdonell, Jackson, *et al.*, 2010; Kazis *et al.*, 2006; Reutens and Berkovic, 1992; Reutens *et al.*, 1993). There is conflicting evidence concerning the cSP. It was increased in some studies (Joo *et al.*, 2008; Macdonell *et al.*, 2001; Tataroglu *et al.*, 2004), but normal in the majority of studies, regardless of the medication status of participants (Badawy *et al.*, 2007; Cantello *et al.*, 2006; Ertas *et al.*, 2000; Groppa *et al.*, 2008; Klimpe *et al.*, 2009). SICI and LICI were normal in three relatively small studies (Cantello *et al.*, 2006; Delvaux *et al.*, 2001; Joo *et al.*, 2008), but most studies showed reduced inhibition (Badawy and Jackson, 2012; Badawy *et al.*, 2006, 2007; Badawy,

Jackson, *et al.*, 2012; Badawy, Macdonell, Berkovic, *et al.*, 2010; Badawy, Macdonell, *et al.*, 2012; Klimpe *et al.*, 2009; Molnar *et al.*, 2006; Münchau *et al.*, 2005). Eight of these studies were conducted by one group. It is unclear whether the data overlap from study to study (see **chapter 7.4**).

Table 2.4: TMS measures in generalised epilepsy.

Study	epilepsy type	N	AED	rMT	aMT	cSP	SICI	LICI	ICF
Aguglia *et al.*, 2000	IGE with versive seizures	10	Y	↑					
	IGE (7JME)	13	Y	↑					
Badawy *et al.*, 2006	IGE (5JME)	15	N	↔					
	IGE + sleep deprivation		N	↔			↓	↓*	↑
Badawy *et al.*, 2007	IGE (11JME)	35	N	↔		↔	↓	↓*	↑
Badawy, Macdonell, *et al.*, 2009b	IGE morning	10	N	↔			↓	↓	↑
	IGE afternoon		N	↔			↓	↓	↑
Badawy, Macdonell, *et al.*, 2009a	IGE preictal	23	N	↓^			↓^	↓^	↑^
	IGE postictal		N	↑^			↑^	↑^	↓^
Badawy, Macdonell, Berkovic, *et al.*, 2010	IGE	59	N	↓			↓	↓**	↑
	IGE + AED		Y	↑¥			↑	↑	↓
Badawy, Macdonell, et al., 2012	Lennox-Gastaut	18	Y	↑			↑	↑	↓
	IGE (refractory)	20	Y	↔			↓↓	↓↓	↑
Badawy, Jackson, *et al.*, 2012	IGE (4 JME)	13	N	↔			↓	↓*	↑
Badawy and Jackson, 2012	IGE	28	N	↔			↓	↓*	↑
Brodtmann *et al.*, 1999	IGE	7	N	↔				↓***	
Cantello *et al.*, 2006	IGE before AED	8	N	↔		↔	↔		↑
	IGE 12 weeks VPA		Y	↔		↔	↔		↔
Delvaux *et al.*, 2001	within 48h of 1st TC seizure	18	N	↑			↔		↓
Ertas *et al.*, 2000	IGE	10	N			↔			
Groppa *et al.*, 2008	IGE - PPR	12	N/Y	↑		↔			
	IGE + PPR	13	N/Y	↔		↔			
Joo *et al.*, 2008	IGE	15	N	↔		↑	↔		↔
	IGE + Zonisamide		Y	↔		↑	↔		↔
Kazis *et al.*, 2006	IGE (7JME)	30	N	↓					
	IGE (7JME) VPA 4 weeks		Y	↔		↓			
	IGE (7JME) VPA 25 weeks		Y	↔		↓			
Klimpe *et al.*, 2009	IGE	15	N	↔	↔	↔	↓		
Macdonell *et al.*, 2001	IGE (9JME)	21	N	↔		↑			

Table 2.4 (Continued)

Study	epilepsy type	N	AED	rMT	aMT	cSP	SICI	LICI	ICF
Molnar *et al.*, 2006	IGE + DBS off	5	Y	↑	↑		↔	↓↓	↔
	IGE + DBS on		Y	↑	↑		↔	↓	↔
	IGE + DBS cyclic		Y	↑	↑		↔	↓	↔
Münchau *et al.*, 2005	IGE + depression - mirtazapine	7	Y	↔	↑	↔	↓		
	IGE + depression + mirtazapine 3 weeks		Y	↔	↓	↔	↔		
	controls + 1 dose mirtazapine		N	↔	↔	↑	↔		
Reutens & Berkovic, 1992	IGE - AED	11	N	↓					
	IGE + AED	34	Y	↑					
Reutens *et al.*, 1993	IGE - AED	20	N	↓					
	IGE + AED	36	Y	↑					
Tataroglu *et al.*, 2004	IGE	50	N/A	↑		↑			

** at 250ms.** at 150 and 250ms. ***at 175 and 250 ms. ^compared to interictal state. ¥ correlated with seizure freedom after 1 year of treatment. IGE: idiopathic generalised epilepsy (old classification), JME: juvenile myoclonic epilepsy, AED: anti-epileptic drugs, VPA: valproic acid, TC: tonic-clonic, PPR: photoparoxysmal response, DBS: deep brain stimulator, N/Y: included people with and without medication. N/A: information on medication not available, rMT: resting motor threshold, cSP: cortical silent period, SICI: short-interval intracortical inhibition, LICI: long-interval intracortical inhibition, ICF: intracortical facilitation. All data are compared to healthy controls. If no control group was included, this is stated.*

ICF was increased in most studies (Badawy and Jackson, 2012; Badawy *et al.*, 2006, 2007; Badawy, Jackson, *et al.*, 2012; Badawy, Macdonell, Berkovic, *et al.*, 2010; Badawy, Macdonell, *et al.*, 2012; Cantello *et al.*, 2006), and normal in two studies including participants with and without medication (Joo *et al.*, 2008; Molnar *et al.*, 2006). Cortical recovery curves display the Test Response/Conditioned Response ratio (TR/CR) plotted against the range of interstimulus intervals. In these studies, people with IGE displayed a peak at interstimulus interval 250ms, where the TR/CR was around 200%, compared to 100% in healthy subjects. People with IGE also displayed a smaller peak, at an interstimulus interval of 150 ms. Cortical recovery curves have not been widely studied by other authors, but appear to provide valuable additional information. The pathophysiologic significance of the facilitation or decrease in inhibition in people with epilepsy has not yet been elucidated. The fact that it is maximal at a long interstimulus interval suggests defective GABA-B inhibition as one

of the possible mechanisms (Badawy and Jackson, 2012; Badawy *et al.*, 2006; Badawy, Macdonell, *et al.*, 2009a). Further study, especially of the recovery curve and the long interstimulus intervals, is needed to confirm these results and understand the underlying mechanisms. In Lennox-Gastaut syndrome, a severe form of epilepsy characterised by frequent and refractory seizures and mental handicap, the balance tilts towards inhibition, reflected by an increased rMT, increased SICI and LICI, and reduced ICF (see table 2.4) (Badawy, Macdonell, *et al.*, 2012). In this study, an attempt was made to correct for the use of medication by comparing people with Lennox-Gastaut who used two or more AEDs, with people suffering from other types of refractory epilepsy who also used two or more AEDs. The authors corrected for peri-ictal changes in excitability by ensuring seizure freedom around the TMS measurements (Badawy, Macdonell, *et al.*, 2012). It remains unclear why this condition shows hypoexcitability, whereas other types of epilepsy show cortical hyperexcitability.

Juvenile Myoclonic Epilepsy (JME) and progressive myoclonic epilepsy were often studied separately from other forms of genetic epilepsies. JME is one of the most common forms of genetic epilepsy (Gardiner, 2005). For this reason, and to improve readability of the results, I have chosen to keep JME and other forms of epilepsy with myoclonic seizures separate in this review. In people with JME, the rMT seems to be normal or increased, although most studies have been carried out in people taking medication (see table 2.5) (Akgun *et al.*, 2009; Badawy, Macdonell, Jackson, *et al.*, 2010; Brown *et al.*, 1996; Canafoglia *et al.*, 2010; Caramia *et al.*, 1996; Danner *et al.*, 2009; Manganotti *et al.*, 2001, 2004, 2006; Pfütze *et al.*, 2007; Valzania *et al.*, 1999). The only study in which people with JME did not take AEDs showed a normal rMT (Badawy, Macdonell, *et al.*, 2009b). In people with JME most studies, except two, showed decreased inhibition reflected by lower SICI and LICI (Badawy, Macdonell, Jackson, *et al.*, 2010; Canafoglia *et al.*, 2010; Caramia *et al.*, 1996; Hanajima *et al.*, 2008; Manganotti *et al.*, 2006; Turazzini *et al.*, 2004). One study found no differences in excitability between patients and healthy controls (Pfütze *et al.*, 2007). It is argued that this may be due to the fact that the patients were on AEDs. In the studies that showed decreased SICI, patients were on AEDs. Circadian rhythms were taken into account by measuring once in the evening and once in the morning, but seizure occurrence around TMS measurements is not mentioned (Pfütze *et al.*, 2007). Another study only found reduced SICI in people with generalised myoclonus (Brown *et al.*, 1996). Decrease of SICI and LICI was more marked in people with JME after sleep deprivation

(Manganotti *et al.*, 2006). One study found decreased ICF in people with JME taking medication and one found decreased ICF in people with Lafora body progressive myoclonic epilepsy taking medication (Canafoglia *et al.*, 2010; Manganotti *et al.*, 2006). Another study showed that in people with progressive myoclonic epilepsy taking medication, SICI and LICI decrease was more pronounced than in people with JME on medication (Badawy, Macdonell, Jackson, *et al.*, 2010).

Studies investigating the rMT in drug-naïve people with IGE (old classification) were compared in a meta-analysis (Brigo, Storti, Benedetti, *et al.*, 2012). Data on JME were extracted from studies that included people with different types of IGE. It suggested that the rMT was significantly lower in drug-naïve people with JME (40 people) than in controls (N=161). In people with types of IGE other than JME, however, the rMT did not significantly differ from controls (41 with IGE, 130 controls).

The most consistent finding in people with JME is reduction in SICI (and LICI when studied), despite the use of medication. In other forms of IGE, this finding is less consistent. SICI is probably GABA-A mediated, and impaired GABA-A mediated inhibition is in line with some reports of mutations of GABA-A receptor subunits in cases of JME (Gardiner, 2005).

Focal epilepsy

Inter-hemispheric difference in excitability appears to be crucial in focal epilepsy, and most groups have studied the hemispheres with (ipsilateral) and without (contralateral) the seizure focus separately. Results regarding the rMT are conflicting (see table 2.6). Most studies did not find significantly different rMTs between the hemispheres or between people with or without epilepsy (Badawy and Jackson, 2012; Badawy *et al.*, 2006, 2007; Badawy, Jackson, *et al.*, 2012; Hamer *et al.*, 2005; Klimpe *et al.*, 2009; Nezu *et al.*, 1997; Varrasi *et al.*, 2004; Werhahn, Lieber, *et al.*, 2000). Others found significant differences between ipsi- and contra- lateral rMTs (Badawy *et al.*, 2007; Badawy, Macdonell, Berkovic, *et al.*, 2010; Kim *et al.*, 2008). In studies in participants on anti-epileptic medication no difference was found between the hemispheres, but the rMT was found to be increased bilaterally compared to controls (Badawy, Macdonell, Berkovic, *et al.*, 2010; Cantello *et al.*, 2000; Cicinelli *et al.*, 2000; Cincotta *et al.*, 1998; Hufnagel, Elger, Ising, *et al.*, 1990; Hufnagel, Elger, Marx, *et al.*, 1990; Tataroglu *et al.*, 2004).

The cSP was normal in most studies, and regardless of the medication status of participants (Cantello *et al.*, 2000, 2006; Joo *et al.*, 2010; Varrasi *et al.*, 2004; Werhahn, Lieber, *et al.*, 2000). Two studies found a prolonged cSP (Cincotta *et al.*, 1998; Kim *et al.*, 2008), while three studies found a shorter cSP, especially ipsilaterally (Cicinelli *et al.*, 2000; Hamer *et al.*, 2005; Hattemer *et al.*, 2006). The cSP was also reduced in post-stroke epilepsy (Kessler *et al.*, 2002) but this was not confirmed by other studies (Kim *et al.*, 2008; Turazzini *et al.*, 2004).

SICI was found to be decreased in people with focal epilepsy, especially ipsilaterally (Badawy and Jackson, 2012; Badawy *et al.*, 2006, 2007; Badawy, Jackson, *et al.*, 2012; Badawy, Macdonell, Berkovic, *et al.*, 2010; Badawy, Macdonell, *et al.*, 2009a, 2009b, 2012; Varrasi *et al.*, 2004). All but three of these studies are from the same group, and were mainly conducted in drug-naïve people. In these studies, ICF was increased ipsilaterally (Badawy and Jackson, 2012; Badawy *et al.*, 2006, 2007; Badawy, Jackson, *et al.*, 2012; Badawy, Macdonell, Berkovic, *et al.*, 2010; Badawy, Macdonell, *et al.*, 2009a, 2009b, 2012; Cantello *et al.*, 2000; Kim *et al.*, 2008; Varrasi *et al.*, 2004). Other studies, some of which were also conducted in drug-naïve people, found no difference in SICI (bilaterally) or ICF between people with focal epilepsy and healthy controls (Cantello *et al.*, 2006; Hamer *et al.*, 2005; Hattemer *et al.*, 2006; Joo *et al.*, 2010; Kim *et al.*, 2008; Klimpe *et al.*, 2009; Turazzini *et al.*, 2004; Werhahn, Lieber, *et al.*, 2000). One study found decreased ICF ipsilaterally (Werhahn, Lieber, *et al.*, 2000). The cortical recovery curves of people with focal epilepsy also shows a peak at an interstimulus interval of 250ms that is somewhat smaller than in people with IGE (old classification) (Badawy and Jackson, 2012; Badawy *et al.*, 2006; Badawy, Macdonell, *et al.*, 2009a). The peak at an interstimulus interval of 150ms, which is seen in people with IGE, is absent in people with focal epilepsy. In summary, although not unequivocal, the findings in people with focal epilepsy seem to point towards hyperexcitability of the hemisphere ipsilaterally to the epileptic focus, especially when considering the studies of Badawy *et al* (Badawy and Jackson, 2012; Badawy *et al.*, 2006, 2007; Badawy, Jackson, *et al.*, 2012; Badawy, Macdonell, Berkovic, *et al.*, 2010; Badawy, Macdonell, *et al.*, 2009a, 2009b, 2012).

Table 2.5: TMS measures of cortical excitability in epilepsies with myoclonic seizures.

Study	epilepsy type	N	AED	rMT	aMT	MEP	cSP	SICI	LICI	ICF
Akgun *et al.*, 2009	JME	21	Y	↑		↔	↑			
	asymptomatic siblings of people with JME	21	N	↑		↔	↑			
Badawy,Macdonell, *et al.*, 2009b	JME morning	10	N	↔				↓↓	↓↓	↑↑
	JME afternoon		N	↔				↓	↓	↑
Badawy, Macdonell, Jackson,*et al.*, 2010	PME	6	Y	↔				↓↓↓	↓↓↓	
	JME refractory	9	Y	↔				↓↓	↓↓	
	JME well-controlled	10	Y	↔				↓	↓	
Brown *et al.*, 1996	Cortical Myoclonus (generalised jerks)	8	Y	↑				↓		
	Cortical Myoclonus (focal jerks)	10	Y	↑↑				↔		
	Epilepsy + cortical myoclonus	9		↑				↔		
Canafoglia *et al.*, 2010	Unverricht-lundborg disease	10	Y	↔	↑	↔		↓	↔	↔
	Lafora body disease	5	Y	↔	↑	↔		↓	↓	↓
Caramia *et al.*, 1996	JME	7	Y					↓		
Danner *et al.*, 2009	Uverricht-Lundborg disease	24	Y	↑	↑*		↑**			
Hanajima *et al.*, 2008	Benign myoclonus epilepsy n.o.s.	11	Y		↔	↔		↓		
Manganotti *et al.*, 2004	JME	9	Y	↔		↔		↓		↔
Manganotti *et al.*, 2006	JME	10	Y	↔		↔		↓		↓
	JME+ sleep deprivation		Y	↑		↔	↔	↓↓		↔
Pfütze *et al.*, 2007	JME morning	12	Y	↔				↔		↔
	JME evening		Y	↔				↔		↔
Tataroglu *et al.*, 2004	Myoclonic epilepsy n.o.s.	12	N/A	↓			↑			
Valzania *et al.*, 1999	PME	12	Y	↔			↔		↓	↑***

in abductor digiti minimi not abductor pollicis brevis. ** in controls: decrease with age, not in patient group. * at 50 ms. JME: juvenile myoclonic epilepsy. PME: progressive myoclonic epilepsy. AED: anti-epileptic drugs. N.o.s.: not otherwise specified. N/Y: included people with and without medication. N/A: information on medication not available. rMT: resting motor threshold. MEP: Motor evoked potential. cSP: cortical silent period. SICI: short-interval intracortical inhibition. LICI: long-interval intracortical inhibition. ICF: intracortical facilitation. BECT: benign childhood epilepsy with centro-temporal spikes. All data are compared to healthy controls. If no control group was included, this is stated.*

Table 2.6: TMS measures of cortical excitability in focal epilepsy.

Study	epilepsy type	N	AED	rMT		aMT		MEP		cSP		SICI		LICI		ICF	
				i	c	i	c	i	c	i	c	i	c	i	c	i	c
Badawy *et al.*, 2006	Focal	15	N	↔	↔							-		-		-	
	Focal + sleep deprivation	15	N	↔	↔							↓	↔	↓	↓^	↑	↔
Badawy *et al.*, 2007	Focal	27	N	↔	↓							↓	↔	↓	↔	↑	↔
Badawy, Macdonell, *et al.*, 2009b	Focal morning	10	N	↔	↔							↓		↓ ^		↑	
	Focal afternoon		N	↔	↔							↔		↔		↔	
Badawy, Macdonell, *et al.*, 2009a	Focal preictal	35	N	↓	↔							↓	↔	↓↓	↔	↑	↔
	Focal postictal		N	↑	↑							↑↑	↔	↑↑	↔	↓	↔
Badawy, Macdonell, Berkovic, *et al.*, 2010	Focal	47	N	↑	↓							↓	↔	↓↓	↔	↑	↔
	Focal + AED		Y	↑	↑							↔	↔	↔	↔	↔	↔
Badawy, Macdonell, *et al.*, 2012	Focal (refractory) 3 AED	20	Y	↑								↓		↓ ↓		↑	
Badawy, Jackson, *et al.*, 2012	Focal	11	N	↔	↔							↓	↔	↓	↔	↑	↔
Badawy and Jackson, 2012	Focal	22	N	↔								↓		↓		↑	
Cantello *et al.*, 2000	Cryptogenic partial	18	Y	↔	↔					↔	↔	↓	↔	-		↑	↔

i: hemisphere ipsilateral to epileptic focus. c: contralateral to epileptic focus. ^ at 250 ms. rMT: resting motor threshold. MEP: Motor evoked potential. cSP: cortical silent period. SICI: short-interval intracortical inhibition. LICI: long-interval intracortical inhibition. ICF: intracortical facilitation. VPA: valproic acid

Table 2.6 (continued)

Study	epilepsy type	N	AED	rMT		aMT		MEP		cSP		SICI		LICI		ICF	
				i	c	i	c	i	c	i	c	i	c	i	c	i	c
Cantello *et al.*, 2006	Focal before VPA	7	N	↔	↔					↔	↔	↔	↔			↔	↔
	Focal 12 wk VPA		Y	↑	↑					↔	↔	↔	↔			↔	↔
Cicinelli *et al.*, 2000	Cryptogenic focal	16	Y	↑	↑					↓	↔	-		-		-	
Cincotta *et al.*, 1998	Partial myoclonic	8	Y	↑	↑					↑ *	↑						
	Partial non-myoclonic	10	Y	↔	↔					↔	↔						
Hamer *et al.*, 2005	Focal 2AED *no controls*	23	Y	↔	↔					↓	↔	↔	↔			↔	↔
Hattemer *et al.*, 2006	Focal catamenial	6	Y	↔	↔					↓ #	↔	↔	↔				
Hufnagel, Elger, Marx, *et al.*, 1990	Focal (TLE) + AED	18	Y	↑	↑												
	Focal (TLE) - AED		N	↑	↑												
Hufnagel, Elger, Ising, *et al.*, 1990	Focal (TLE)	53	Y	↑	↑		↔	↔									
Joo *et al.*, 2010	Focal - zonisamide	24	N	*no*	*controls*			↔	↔	↔	↔	↔	↔			↔	↔
	Focal + zonisamide		Y	↔	↔			↓	↔	↔	↔	↔	↔			↔	↔
Kessler *et al.*, 2002	Post-stroke focal *no controls*	6	N	↔	↔			↓	↔	↓	↔						
Kim *et al.*, 2008	Post-stroke focal *no controls*	18	Y	↑	↔			↔ *	↓ *	↑	↑	↔	↔			↑ *	↓ *

*TLE: temporal lobe epilepsy. *increased interhemispheric difference. #for luteal phase and menstruation.*

Table 2.6 (continued)

Study	epilepsy type	N	AED	rMT		aMT		MEP		cSP		SICI		LICI		ICF	
				i	c	i	c	i	c	i	c	i	c	i	c	i	c
Klimpe *et al.*, 2009	Focal	10	N	↔ $		↔ $				↔ $		↔ $					
Kotova & Vorob'eva, 2007	Focal - AED	13	N	↓ $													
	Focal + AED	20	Y	↔ $													
Manganotti *et al.*, 1999	Partial - AED	6	N	*no controls*													
	Partial + 5 weeks AED		Y	↑ %		↑ %		↔%		↔%							
Nezu *et al.*, 1997	BECT - AED	5	N	↔													
	BECT + AED	8	Y	↑													
Tataroglu *et al.*, 2004	Partial	48	Y	↑	↑					↔	↔						
Turazzini *et al.*, 2004	Post-stroke focal - AED	8	N	*no controls*													
	Post-stroke focal CBZ		Y	↑ %		↑ %		↔ %		↔% ↔%		↔% ↔%				↔ %	
Varrasi *et al.*, 2004	Focal	21	N	↔	↔					↔	↔	↓	↔			↔ R ↑ L	
Werhahn, Lieber, *et al.*, 2000	Focal -AED	15	N	↔	↔					↔	↔	↔	↓			↓↓	↓
Wright *et al.*, 2006	refractory TLE	18		*no controls*								↑**				↓**	

*$ only dominant hemisphere studied regardless epilepsy side. **peri-ictally, correlated positively with seizure occurrence within 48hours. % side not specified. AED: anti-epileptic drugs. BECT: benign childhood epilepsy with centro-temporal spikes. CBZ: carbamazepine. TLE: temporal lobe epilepsy. R: right hemisphere, L: left hemisphere. rMT: resting motor threshold. MEP: Motor evoked potential. cSP: cortical silent period. SICI: short-interval intracortical inhibition. LICI: long-interval intracortical inhibition. ICF: intracortical facilitation. All data are compared to healthy controls. If no control group was included, this is stated.*

2.2.4 TMS-EEG in epilepsy

The combination of TMS with EEG is relatively novel (Ilmoniemi and Kicić, 2010; Lioumis *et al.*, 2009). In epilepsy, two types of approaches can be identified: provoking epileptiform discharges, or quantification of the TMS-evoked potential (TEP) in the EEG. In a study following the first type of approach, the EEG patterns of people with focal epilepsy were assessed after TMS and compared with those of healthy controls (Valentin *et al.*, 2008). Two phenomena were seen exclusively in 11 of the 15 people with epilepsy: in three people, stimulation of the epileptogenic area triggered a delayed response with spikes and sharp waves, sometimes similar to the person's epileptiform discharges seen on a diagnostic EEG recording. In nine people, including one showing a delayed response, extra-temporal TMS triggered a new rhythm on the EEG that differed from the background EEG rhythm. Both of these phenomena were correlated with seizure lateralisation. Another study showed that during an epileptic discharge, there is an increase in information flow from the epileptic focus to other areas of the epileptogenic region (Kimiskidis *et al.*, 2013). When TMS was applied after the start of the epileptic discharge, however, this flow was reduced. Application of a short train of TMS stimuli at a frequency of 3-5Hz just after the start of epileptic discharges significantly shortened the duration of epileptic discharges (Kimiskidis *et al.*, 2013). In a study of people with progressive myoclonic epilepsy, it was shown that the power of oscillations in the alpha, beta, and gamma band of the EEG upon TMS was lower in people with progressive myoclonic epilepsy than in controls. People with progressive myoclonic epilepsy showed less synchronisation in the alpha and beta bands than controls. The P30 response (the positive peak around 30ms after stimulation) was increased in people with progressive myoclonic epilepsy, which was interpreted as a sign of hyperexcitability. The N100/P180 peak was decreased in people with progressive myoclonic epilepsy potentially indicating defective inhibition (Julkunen *et al.*, 2013). The late EEG response to TMS was increased in amplitude after sleep deprivation, but more in people with JME than in controls (Del Felice *et al.*, 2011). Using TMS-EEG, covert, preictal states of the brain could be identified (Kimiskidis *et al.*, 2015). TMS-EEG may be valuable for studying brain connectivity in epilepsy (Kimiskidis *et al.*, 2013; Manganotti and Del Felice, 2013; Rogasch and Fitzgerald, 2012). In periventricular nodular heterotopia, a developmental condition that causes epilepsy and reading disability in people with normal intelligence, the geographic distribution of the late response to TMS (>225ms after the stimulus) revealed aberrant connectivity patterns in the people with periventricular nodular heterotopia (Shafi *et al.*, 2015). A recent study showed that the TEP amplitude

correlated with melatonin and cortisol levels in healthy, sleep-deprived controls (Ly *et al.*, 2016). This may prove to be particularly important in the context of epilepsy, especially as other recent studies linked seizures and interictal epileptiform discharges to cortisol (van Campen *et al.*, 2015, 2016).

It is clear that TMS-EEG holds great potential for application in epilepsy, but questions remain, especially on how to deal with stimulation artefacts and how to quantify the TMS-EEG responses. In **chapter 8,** I describe the results of my own TMS-EEG study in people with JME, migraine, and healthy controls, where a different approach is taken to quantify TMS-EEG responses.

2.2.5 Potential clinical application of diagnostic TMS

Prediction of AED response

A potential clinical application of TMS is the prediction of the outcome(s) of AED treatment. On group level, AEDs have been consistently shown to affect cortical excitability (Kazis *et al.*, 2006; Münchau *et al.*, 2005; Nezu *et al.*, 1997; Reutens and Berkovic, 1992; Reutens *et al.*, 1993). Treatment with AEDs increases the rMT, even if this was low or normal prior to treatment (Kazis *et al.*, 2006; Münchau *et al.*, 2005; Nezu *et al.*, 1997; Reutens and Berkovic, 1992; Reutens *et al.*, 1993), although in one study an AED failed to increase the MT significantly (Joo *et al.*, 2008). This was probably due to the fact that zonisamide was used in this study, whereas the others included sodium valproate, carbamazepine, and lamotrigine. Zonisamide has multiple modes of action, which are complex and may not directly affect MT (Biton, 2007). Significant increase of the rMT to above normal level after the initiation of AED treatment has been positively correlated with seizure reduction (and freedom) after one year (Badawy, Macdonell, Berkovic, *et al.*, 2010). This opens up the possibility for personalised treatment in epilepsy. So far, treatment decisions in epilepsy are made mostly on empirical grounds. With TMS, reliable assessment of the inhibition/excitation balance may be possible and the effect of medication may be assessed shortly after treatment initiation. TMS may thus have the potential to guide decision-making in the treatment of epilepsy. It could be of use in determining the doses needed, or it could assist in choosing the right drug. Several doses or drugs could be tried sequentially in a person with epilepsy, and instead of awaiting the clinical effect on the number of seizures for months, excitability could be tested after several weeks. A substantial decrease in excitability may mean that the drug is effective. TMS may also be used to study the temporal dynamics of cortical excitability in response to AED or candidate drugs. Further

research is warranted to assess TMS reliability on an individual, rather than group, basis and to determine what magnitude of decrease in cortical excitability is associated with seizure freedom.

Pre- and post- epilepsy surgery evaluation using cortical excitability

Focal epilepsy can sometimes be treated by surgically removing the epileptic focus. Cortical excitability measured with TMS, pre- and post- operatively, may help predict the surgical outcome (Kamida *et al.*, 2007; Läppchen *et al.*, 2008). A decrease in excitability in the epileptic hemisphere has been correlated with a significant seizure reduction (mean follow-up 16 months). One person, who did not show a reduction in excitability, had a suboptimal post-operative outcome (Kamida *et al.*, 2007). These results were replicated in the non-epileptic hemisphere (Karadaş *et al.*, 2011; Läppchen *et al.*, 2008). Epilepsy surgery seems to change interhemispheric inhibitory interactions between the motor cortices (Läppchen *et al.*, 2011). A case report discussed two people with cerebral tumours (glioblastoma multiforme WHO IV and metastasis) and focal motor seizures, in whom TMS showed a loss of SICI and strongly increased facilitation. The cSP was normal in both (Irlbacher *et al.*, 2002). Another report on two people with meningioma and simple and complex partial seizures showed lengthening of the cSP in the person with simple partial seizures. Post-surgically, the cSP returned to normal levels in this individual with no cSP changes seen in the other (Cincotta *et al.*, 2002). Hyperexcitability was shown to normalise after successful subpial transection in an individual with simple partial seizures (Shimizu *et al.*, 2001). One of the issues in the post-operative management of people with epilepsy is the subsequent treatment with AEDs. Often AED treatment is continued until at least one year after the operation, and some people will never stop taking AEDs in fear of new seizures. TMS could help decision-making in the post-operative phase, and may help differentiate between the people who are at risk of relapse and who should continue taking AEDs and those who are likely to stay seizure free without medication.

Prediction of seizure susceptibility

There is some evidence that cortical excitability significantly rises in the 24 hours preceding a seizure, reflected by lower SICI and LICI and higher ICF (Badawy, Macdonell, *et al.*, 2009a; Wright *et al.*, 2006). Twenty-four hours postictally, excitability is lower than interictally (Badawy, Macdonell, Berkovic, *et al.*, 2010; Delvaux *et al.*, 2001). TMS could, theoretically, help predict seizure occurrence, for example in the setting of pre-surgical video-EEG evaluation. In

this setting, time is limited, and ictal recordings are necessary to accurately determine the epileptic source. To increase the likelihood of a seizure that can be recorded, AEDs are tapered. TMS could guide clinicians in decisions regarding the doses of medication. Before this is possible further research is needed to demonstrate that the preictal rise of excitability is significant in individuals and not only on group level. TMS is probably not a technique that could be used for seizure prediction in an out-patient setting, but it has potential in a clinical environment.

Inter- and intra- individual variability of TMS measures

Before TMS can be implemented in clinical settings for the above purposes, the inter- and intra- individual variability of the measures related to cortical excitability has to be understood. Early studies, for example, show that the motor threshold varies considerably between individuals (Mills and Nithi, 1997; Wassermann, 2002). The intra-individual variability was relatively low (Kimiskidis et al., 2004; Koski et al., 2005). The motor threshold did not differ significantly between people with generalised epilepsy and controls, except in JME (Brigo, Storti, Benedetti, *et al.*, 2012). SICI and intracortical facilitation (ICF), measured using paired-pulse protocols, were also variable between individuals (Boroojerdi *et al.*, 2000; Cahn *et al.*, 2003; Cicinelli *et al.*, 2000; Inghilleri *et al.*, 1990; Kujirai *et al.*, 1993; Maeda *et al.*, 2002; Nakamura *et al.*, 1997; Orth *et al.*, 2003; Wassermann, 2002; Ziemann *et al.*, 1996). In two studies, the intra-individual variability of SICI was between 31% and 37%, and the interindividual variability was between 44% and 67%. ICF inter-individual variability was lower (21% and 23%), but intra-individual variability was high (22% and 60%) (Boroojerdi et al., 2000; Orth et al., 2003). LICI was studied less extensively than SICI and ICF, but results appear to point to a similar variability (Du et al., 2014; Lang et al., 2011; Nakamura et al., 1997; O'Leary et al., 2015; Opie and Semmler, 2014a, 2014b; Valls-Solé et al., 1992). Few report LICI recovery curves with more than four interstimulus intervalss (Du et al., 2014; Lang et al., 2011; Nakamura et al., 1997; Valls-Solé et al., 1992). The shape of this curve differs depending on the protocols used, but all show inhibition around 100-150ms interstimulus intervals. One study showed that curves shapes are to some extent reproducible in the same individual (Du et al., 2014), but the inter-individual variability, studied in relatively small cohorts, is large (Du et al., 2014; Lang et al., 2011; Nakamura et al., 1997; Valls-Solé et al., 1992). As discussed in the previous sections, SICI and LICI recovery curves were extensively studied in different forms of epilepsy, and not only did the reported shape and absolute values of the curves consistently differ between groups of people with epilepsy and healthy controls, but the curves also

differed according to medication use, type of epilepsy, time-relation to seizures and refractoriness of the seizures to medication (Badawy and Jackson, 2012; Badawy *et al.*, 2007; Badawy, Vogrin, *et al.*, 2013a, 2013b, 2013c; Badawy *et al.*, 2014; Badawy, Jackson, *et al.*, 2012, 2013; Badawy, Macdonell, Berkovic, *et al.*, 2010; Badawy, Macdonell, *et al.*, 2009a, 2009b, 2012; Badawy, Macdonell, Jackson, *et al.*, 2010). Based on these studies, SICI and LICI recovery curves are promising candidates as biomarkers for epilepsy. Whether they can be used as such on an individual level depends on the inter- and intra- individual variability of these curves.

2.2.6 Summary and conclusion

Responses to TMS are fairly stable. Even when using a coil with focused beam, TMS typically recruits a large ensemble of neuronal cells, including neurons with excitatory and inhibitory properties. It is plausible that stimulating a relatively large area results in a consistent net response, while in some cases of direct cortical stimulation, variability in the response may be due to insufficient neuronal recruitment (Lesser *et al.*, 2008). Research into cortical excitability, spurred by the development of TMS, suggests that cortical excitability is a dynamic feature of the human brain influenced by internal and external factors. Imbalance of inhibitory and excitatory factors seems to be important in the development of epilepsy. People with epilepsy (apart from those with Lennox-Gastaut syndrome) have a hyperexcitable cortex compared to healthy subjects. AEDs have an important role in reducing this cortical hyperexcitability.

Cortical excitability may be used as a marker of disease activity. Single- and paired- pulse TMS protocols have the potential to help reduce the disease burden of epilepsy by predicting the outcome of AED treatment in individuals. Findings are, however, sometimes contradictory, and TMS is not yet ready to be used in clinical practice. The challenge that lies ahead is to investigate whether TMS can assess changes in cortical excitability at an individual level and whether these changes are robust enough to predict treatment outcome. In **chapter 7** I will address this question. More clinical studies that correlate individual changes in cortical excitability to disease activity are necessary. Cortical excitability assessments may also be valuable for the early prediction of post-operative outcomes as well as for decision-making regarding the post-operative continuation of AED treatment. Prospective studies are needed to investigate the predictive power of pre- and post- operative cortical excitability. As will be shown in **chapter 8,** TMS-EEG offers exciting new opportunities to study the key features of the epileptic cortex. Future research will, without doubt, further develop this technique. One

prospect is the possibility of assessing connectivity in epileptic circuits, offering insights into pathophysiology. In the future, this information may help to guide and evaluate epilepsy surgery, and aid the development of new therapies for the condition.

3

Meta-analysis of the co-occurrence of migraine and epilepsy

"It is fair to say that, in general, no problems have been exhausted;
Instead, men have been exhausted by the problems."[3]

3.1 Introduction

As I have described in **chapter 2,** epilepsy is often accompanied by other conditions (Gaitatzis *et al.*, 2012). The comorbidities of epilepsy are a group of medical conditions whose prevalence is increased in people with epilepsy relative to the general population (Gaitatzis *et al.*, 2012; Gaitatzis, Trimble, *et al.*, 2004). One of particular interest is migraine. As described in **chapter 2,** the co-occurrence of epilepsy and migraine has a number of therapeutic, prognostic, and pathophysiological implications. Their co-occurrence may influence the choice of anti-epileptic drug, and also predict a greater probability of treatment failure (Velioglu *et al.*, 2005). A growing number of shared genetic mutations and polymorphisms have been identified (Haan *et al.*, 2008; Winawer and Connors, 2013). There is evidence suggesting that both migraine and epilepsy are related to abnormal neuronal excitability in the cerebral cortex (Badawy and Jackson, 2012; Dreier *et al.*, 2012; Rogawski, 2008; Winawer and Connors, 2013). A fundamental step in exploring the relationship between migraine and epilepsy is to understand the strength of the comorbid association between these two disorders. Some previous studies have suggested that the prevalence of migraine may be as much as 160% higher in people with epilepsy (Gaitatzis *et al.*, 2012). Others have not shown an association between migraine and epilepsy (Brodtkorb *et al.*, 2008; Jalava and Sillanpää, 1996).

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[3] Ramón y Cajal S., (translation by Swanson N, Swanson LW). *Advice for a young investigator.* The MIT Press, Cambridge, MA, 1999, p14

The aim of the study described in this chapter is to examine the prevalence of migraine in people with epilepsy by carrying out a systematic review and meta-analysis of studies that assessed the prevalence of migraine in people with epilepsy, and the prevalence ratio of migraine in people with epilepsy compared to those without epilepsy. With an appropriately inclusive search strategy, this study simultaneously assessed the prevalence of epilepsy in migraineurs as well as the prevalence ratio of epilepsy in migraineurs compared to people without migraine.

3.2 Methods

3.2.1 Protocol

A protocol was developed according to the PRISMA guidelines, those of the MOOSE group, and the Ottawa Non-Randomized Studies Workshop (Liberati *et al.*, 2009; Reeves *et al.*, 2013; Stroup *et al.*, 2000).

3.2.2 Eligibility criteria

All published studies reporting any measure of the lifetime prevalence of migraine amongst people with epilepsy, or epilepsy amongst migraineurs, were considered eligible for this study. Population-based cohort and case–control studies, both prospective and retrospective, were considered. We chose to limit study eligibility to population-based studies in order to increase the external validity/generalisability of our findings and to mitigate any selection bias.

Eligibility was not limited by any precise definition of migraine although studies that expressly included participants with non-migraine headache types (e.g. tension-type headache or trigeminal autonomic cephalgias) were excluded. Epilepsy was operationally defined as two unprovoked epileptic seizures occurring at least 24 hours apart (Thurman *et al.*, 2011). Migraine and epilepsy were defined as a lifetime history (i.e. lifetime prevalence) based upon the assumption that the shared mechanism between these two disorders is congenital. If more than one study reported data derived from the same study subjects, only the more comprehensive study was included. Study eligibility was not limited by language of publication. Professional colleagues fluent in the appropriate language translated articles when necessary.

3.2.3 Search strategy

The search strategy was designed in consultation with a medical librarian with expertise in systematic reviews. Input was also sought from experts in the fields of epidemiology and epilepsy. The search included the following electronic databases: Ovid MEDLINE (1946 to 2013), PubMed, Ovid EMBASE (1947 to 2013), Web of Science CPCI-S, and PsychInfo. The detailed search strategy is outlined in the Appendix 1. The bibliographies of identified review articles as well as all included studies were manually searched for additional relevant studies. A grey literature search was carried out by manually searching the proceedings of the two most recent (2012-2013) annual meetings of the American Epilepsy Society and the American Academy of Neurology. The last electronic search was performed on 20 December 2013.

3.2.4 Study selection

My colleague Mark Keezer and I independently screened all titles and abstracts identified by the initial search. The full-text of an article was obtained if either of us suspected that it might satisfy the eligibility criteria listed above. The reviewers independently evaluated each full-text article and a final decision was made on whether to include or exclude the study. Any disagreements on study eligibility were settled by consensus.

3.2.5 Data extraction and risk of bias

We independently extracted the data from the primary studies using a data extraction tool specifically designed for this review. Data sufficient to complete a 2x2 contingency table were extracted from each study as well as any reported adjusted effect estimates (prevalence ratio - PR or prevalence odds ratio - POR). Additional data extracted from each study included: study design and source population, sample characteristics, and method of identifying cases of epilepsy and migraine. We contacted two study authors to obtain data not available in the published article. One provided additional data on the migraine status of people with epilepsy (i.e. excluding those with a single unprovoked seizure) (Hesdorffer *et al.*, 2007).

The risk of bias of each included study was independently assessed by MK and I using a quality assessment tool specifically designed for this review but whose design was based upon the recommendations of the Ottawa Non-Randomized Studies Workshop and MOOSE guidelines (Stroup *et al.*, 2000; Wells *et al.*, 2013). The study quality domains included in our tool were: representativeness of the study samples, accuracy of case ascertainment (i.e. of

epilepsy and migraine) and comparability. Both the data extraction and quality assessment tools were piloted on five studies after which adjustments were made.

3.2.6 Data synthesis and analysis

The Wilson method was used to calculate 95% confidence intervals (95%CI) for the lifetime prevalence parameters. We chose not to calculate pooled prevalence estimates after visually inspecting the relevant forest plots, which demonstrated significant heterogeneity. After visually inspecting the forest plots of the PR estimates, and assuming that issues related to the accuracy of migraine status ascertainment would generally be non-differential to epilepsy status (and vice-versa), we calculated pooled PRs using random-effects models as recommended by the Ottawa Non-Randomized Studies Workshop (Valentine and Thompson, 2013). Random-effects models, as opposed to fixed-effects models, produce more conservative pooled estimates, better accommodating the heterogeneity that is frequently seen between observational studies (Valentine and Thompson, 2013). Meta-analyses were carried out to provide more precise PR estimates (i.e. synthetic goal) as well as to measure the impact of different study characteristics on these summary estimates (i.e. analytic goal) (Greenland and O'Rourke, 2008). We chose to primarily pool the unadjusted estimates, when possible, given the risk of additional inter-study heterogeneity that would have been introduced by the differing list of confounders controlled for by individual studies.

We visually inspected forest plots, calculated I^2 ratios (percentage of inter-study variation due to heterogeneity rather than chance) as well as performed subgroup analyses to assess for inter-study heterogeneity (Higgins *et al.*, 2003). There were insufficient data to carry out the planned meta-regressions (subject age, epilepsy aetiology and presence of migrainous aura). The degree of publication bias was evaluated by visual inspection of funnel plots. We did not employ other formal tests to measure the degree of publication bias given that these tools (e.g. Egger's or Begg's test) have not been validated for use in observational studies and the risk of publication bias among observational studies is generally considered to be high (Norris *et al.*, 2013; Stroup *et al.*, 2000). STATA/SE, version 12.0 (StataCorp LP, College Station, Texas, USA) was used to conduct all statistical analyses.

3.3 Results

3.3.1 Study selection

Of the 3,640 de-duplicated records identified during our initial search, the full texts of 121 articles were reviewed (figure 3.1). We included nine articles, one of which described two separate studies, resulting in a total of 10 studies, which comprised a total of 1,548,967 subjects (Baldin *et al.*, 2012; Brodtkorb *et al.*, 2008; Gaitatzis, Carroll, *et al.*, 2004; Jalava and Sillanpää, 1996; Le *et al.*, 2011; Nuyen *et al.*, 2006; Ottman *et al.*, 2011; Russ *et al.*, 2012; Téllez-Zenteno *et al.*, 2005). We later excluded one study. The complex reasoning for this is provided in Table A1 in the appendix.

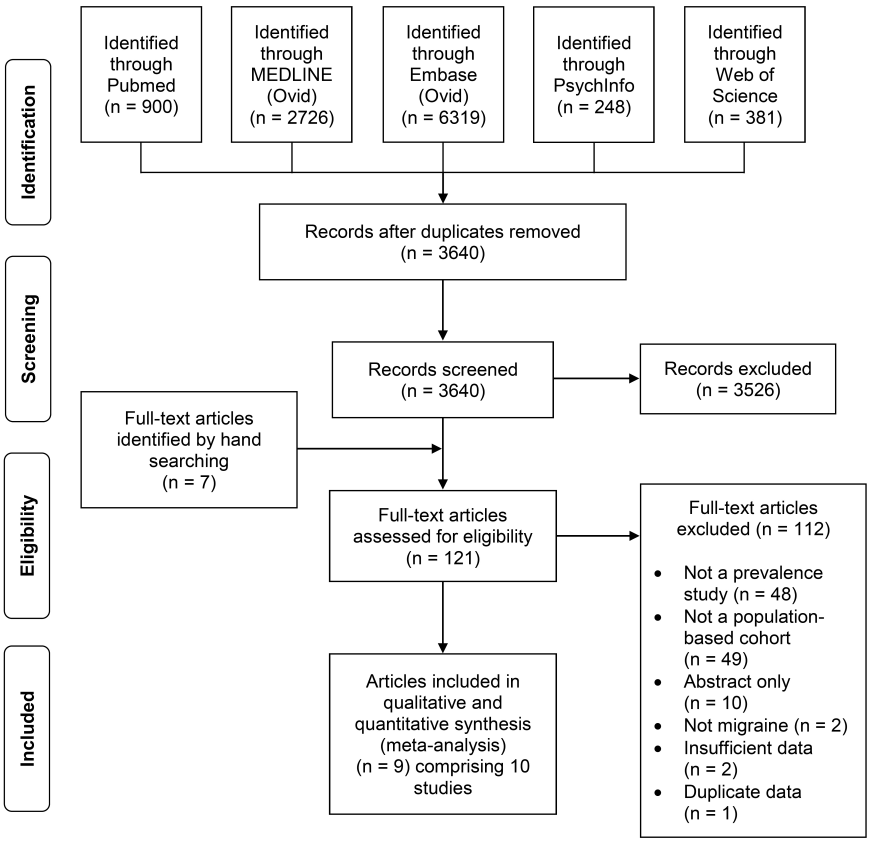

Figure 3.1: PRISMA flow diagram.

3.3.2 Study characteristics and risk of bias assessment

The characteristics of the 10 included studies are presented in Table 3.1, the raw primary data is presented in Table 3.3, and the risk of bias assessment is summarised in Table 3.2. Three of the studies were case-control studies where the sampling frame was defined by a subject's epilepsy status (Hesdorffer *et al.*, 2007; Jalava and Sillanpää, 1996; Ottman *et al.*, 2011). These studies were not used for the estimation of the prevalence of epilepsy among migraineurs or the PR of epilepsy among migraineurs as compared to subjects without migraine. The majority of studies examined adults while only one was primarily of children (Baldin *et al.*, 2012). Two studies had responder proportions below 70% (Jalava and Sillanpää, 1996; Ottman *et al.*, 2011), and two studies did not report the total number of eligible subjects (Gaitatzis, Carroll, *et al.*, 2004; Nuyen *et al.*, 2006).

Table 3.1: Studies included in the meta-analysis.

Study	Country (community)	Study design and source population	Age in years: mean (SD), range	Men (%)	Total sample size (n)	Method of epilepsy diagnosis	Method of migraine diagnosis
Baldin 2012	Iceland (Reykjavik)	Population-based cohort study of all children attending grades 1-10 at almost all public and private schools in the administrative district	10.8 (2.6), NR	49.7	9679[a]	Un-validated single questionnaire item self-administered by a parent	Un-validated multi-item questionnaire algorithm self-administered by a parent
Brodtkorb 2008	Norway (Vågå)	Population-based cohort study of all 18 to 65 year old inhabitants of the Vågå community	35 (NR), 18-65	49.0	1666	Un-validated single questionnaire item administered by MD[b]	Semi-structured headache interview administered by MD
Gaitatzis 2004	UK	Community-based cohort study of persons ≥ 16 years old recruited from 211 participating general practices	≥16 (NR), NR	48.9	1,041,643	Un-validated ICD codes	Un-validated ICD codes
Hesdorffer 2007	Iceland	Population-based case-control study of all individuals in the country with newly diagnosed epilepsy aged >10 years along with two age and sex matched controls	~34 (median) (NR) NR	NR	834	Nationwide surveillance system to identify possible cases of epilepsy (those diagnosed by MD), then confirmed by review of medical records by study nurse	Structured interview although unclear by whom.
Jalava 1996	Finland	Case-control study with multiple methods (hospital/clinic-based and national administrative database) to identify persons with active epilepsy, followed for 32.8 years and then assessed for the purposes of this study	35.6 (NR), 28-45	NR	267	Clinical assessment by study neurologist	Structured interview by study neurologist

Table 3.1 (continued)

Le 2011	Denmark	Population-based cohort study of all persons enrolled in the nation-wide Danish Twin Registry	~44-45 (NR), NR	45.8	31,143	Un-validated self-administered single questionnaire item	Validated self-administered questionnaire (but with poor validity which was not used to correct the prevalence estimates)
Nuyen 2006	Netherlands	Community-based case-control study of persons with epilepsy recruited from 134 participating general practitioners versus those without epilepsy or migraine	42.3 (21.0), NR	49.4	2,730,468	Un-validated ICPC codes	Un-validated ICPC codes
Ottman 2011	USA	Population-based case-control study consisting of a nation-wide random sample of one person ≥18 years old per household	18-65+ (NR), NR	39.8	6976	Validated self-administered single questionnaire item	Un-validated self-administered questionnaire
Téllez-Zenteno 2005a	Canada (NPHS)	Population-based nation-wide cohort study via cluster sampling	30 (median) (NR), NR	49.0	49,026	Un-validated telephone-administered single questionnaire item	Un-validated telephone-administered questionnaire
Téllez-Zenteno 2005b	Canada (CHS)	Population-based nation-wide cohort study via cluster sampling	40 (median) (NR), NR	46.0	130,822	Un-validated telephone-administered single questionnaire item	Un-validated telephone-administered questionnaire

[a] *Although reported by the primary study authors, we did not include febrile seizures in our analyses.* [b] *MD = medical doctor, NR= not reported*

Table 3.2: Primary study data.

Study	E^+M^+ [a]	E^+M^-	E^-M^+	E^-M^-	Adjusted PR (95%CI)[b]	Adjusted POR (95%CI)	Adjustment for possible confounders
Baldin 2012	16	59	1116	8488	2.02 (1.17, 3.51)	NR	Regressed on age, febrile seizure and numerous "recurrent symptoms"
Brodtkorb 2008	9	40	515	1102	NR	NR	NA
Gaitatzis 2004	282	5572	31196	1004593	NR	NR	Effect of age and gender were explored with stratified analyses but no pooled estimates
Hesdorffer 2007	38	149	104	543	1.26 (0.91, 1.77)	NR	Matched case-control design on date of birth and sex
Jalava 1996	18	150	15	84	0.71 (0.37, 1.34)	NR	Matched case-control design on age, sex and domicile
Le 2011	179	354	7597	23013	NR	NR	Effect of presence of aura and gender explored with stratified analyses but no pooled estimates.
Nuyen 2006	21	1238	3046	272616[c]	NR	1.41 (0.73, 2.72)[d]	Matched case-control design on age & sex; multilevel (on general practice) logistic regression, regressed on recent GP contact
Ottman 2011	719	2769	973	2515	1.36 (1.25, 1.48)	NR	Propensity score matched case-control design on age, sex, income, population density, census region, prior head injury, prior stroke and survey panel; EMM adjusted for between survey panel and age, sex as well as severe head injury
Tellez-Zenteno 2005a	43	212	2905	45866	NR	NR	NA
Tellez-Zenteno 2005b	135	598	11851	118238	NR	NR	NA

NA = not applicable; NR = not reported; PR = prevalence ratio; POR = prevalence odds ratio [a] *$E^{+/-}$ = people with or without epilepsy; $M^{+/-}$ = people with or without migraine.* [b] *Reported effect measures describe relative probability (or odds) of migraine among those with epilepsy as compared to those without epilepsy.* [c] *Unmatched data is presented here and used in the analysis.* [d] *The adjusted POR of epilepsy among those with migraine as compared to those without migraine was 1.39 (0.76, 2.54).*

Table 3.3: Bias assessment.

Study	*Representativeness of the study samples*					*Accuracy of case ascertainment*				*Comparability*
	Were those with epilepsy representative of the general population?	**Were those without epilepsy representative of the general population?**	**Were those with migraine representative of the general population?**	**Were those without migraine representative of the general population?**	**Was the response proportion ≥70%?**	**Clear definition of epilepsy?**	**Validated tool to identify people with epilepsy?**	**Clear definition of migraine?**	**Validated tool to identify people with migraine?**	**Confounders controlled for?[a]**
Baldin	Y	Y	Y	Y	Y	N	N	Y	N	Y/N
Brodtkorb	Y	Y	Y	Y	Y	N	N	Y	NR	N/N
Gaitatzis	Y	Y	Y	Y	NR	N	N	N	N	Y[3]/N
Hesdorffer	Y	Y	N	N	Y	Y	NA (MD)	Y	N	Y
Jalava	Y	Y	Y	Y	N	N	NA (MD)	N	NA (MD)	Y
Le 2011[b]	Y	Y	Y	Y	Y	N	N	Y	Y	Y[c]/N
Nuyen	Y	N	Y	N	NR	N	N	N	N	Y/Y
Ottman	Y	Y	Y	Y	N	N	Y	N	N	Y
Tellez-Zenteno	Y	Y	Y	Y	Y	N	N	N	N	N/N
Tellez-Zenteno	Y	Y	Y	Y	Y	N	N	N	N	N/N

Y = yes, N = no, NA = not applicable, NR = not reported, MD = diagnosed by phycisian.

[a] *When applicable, the responses are coded (concerning the prevalence or PR of migraine)/(concerning the prevalence or PR of epilepsy).*

[b] *The source population was a twin registry, therefore the associations between epilepsy and migraine may be inflated.*

[c] *The effect estimates were stratified across potential confounders but the authors did not present any summary estimates.*

Only one study provided a clear definition of epilepsy (Hesdorffer *et al.*, 2007), while four explicitly defined migraine (Baldin *et al.*, 2012; Brodtkorb *et al.*, 2008; Hesdorffer *et al.*, 2007; Le *et al.*, 2011). Six studies relied on un-validated questionnaires to identify cases of epilepsy and/or migraine (Baldin *et al.*, 2012; Brodtkorb *et al.*, 2008; Le *et al.*, 2011; Ottman *et al.*, 2011; Téllez-Zenteno *et al.*, 2005), while two studies similarly relied upon un-validated ICD or ICPC codes (Gaitatzis, Carroll, *et al.*, 2004; Nuyen *et al.*, 2006). Two studies incorporated validated questionnaires for migraine (Le *et al.*, 2011), or epilepsy (Ottman *et al.*, 2011), but neither had sensitivities above 77%. Five studies controlled for potential confounders (Baldin *et al.*, 2012; Hesdorffer *et al.*, 2007; Jalava and Sillanpää, 1996; Nuyen *et al.*, 2006; Ottman *et al.*, 2011).

3.3.3 Prevalence estimates

The lifetime prevalence of migraine among people with epilepsy ranged from 1.7% to 33.6% (figure 3.2A). Those studies that used administrative data and ICD/ICPC codes to identify cases of epilepsy and migraine reported the lowest prevalence estimates (Gaitatzis, Carroll, *et al.*, 2004; Nuyen *et al.*, 2006).

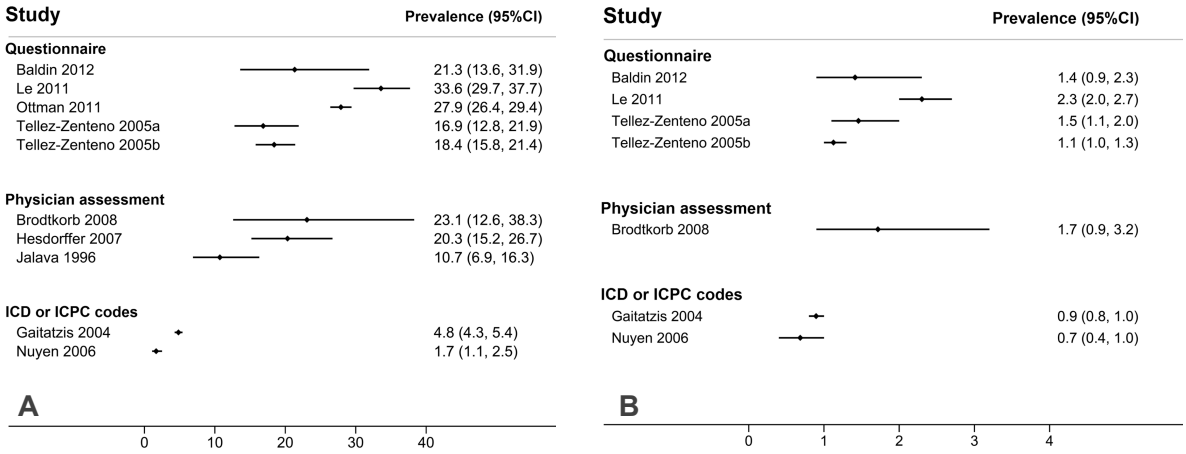

Figure 3.2: Lifetime prevalence of epilepsy and migraine. *A: Lifetime prevalence of migraine in people with epilepsy (%). B: Lifetime prevalence of epilepsy in people with migraine (%).*

The lifetime prevalence of epilepsy among migraineurs ranged from 0.7% to 2.3% (figure 3.2B). Again, those studies that used administrative data and ICD/ICPC codes to identify cases reported the lowest prevalence estimates (Gaitatzis, Carroll, *et al.*, 2004; Nuyen *et al.*, 2006).

3.3.4 Prevalence ratio estimates

Overall, there was a 52% increase in the lifetime prevalence of migraine among people with epilepsy, as compared to those without epilepsy [PR: 1.52 (95%CI: 1.29, 1.79)] (figure 3.3). There was a large degree of heterogeneity between studies, much of which may be explained by the

method of case ascertainment. In those studies where cases of epilepsy and migraine were identified by a physician's assessment, the pooled PR was 0.93 (0.61, 1.41), while it was 1.76 (1.39, 2.24) and 1.60 (1.43, 1.79) when cases were identified with a formal questionnaire or using ICD/ICPC codes.

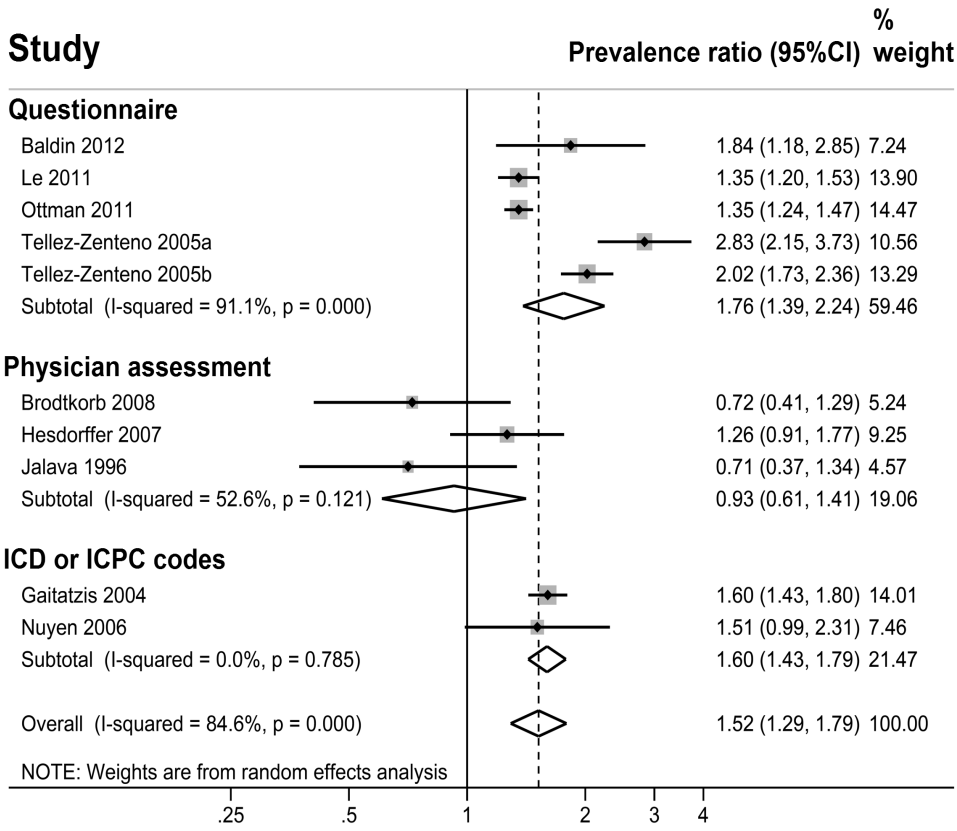

Figure 3.3: Prevalence ratio of migraine in people with epilepsy stratified by case ascertainment method.

Adjustment for potential confounders was also a source of heterogeneity, although not as striking as case ascertainment (figure 3.4). The overall adjusted PR was 1.22 (0.88, 1.56). It is worth noting that this pooled PR crosses the null (i.e. a PR of 1.0) due to one study (Jalava and Sillanpää, 1996). The adjusted PR estimate for Baldin 2012 increased after adjustment rather than decreased (from 1.84 to 2.02). Overall, there was a 79% increase in the lifetime prevalence of epilepsy among migraineurs, as compared to those without migraine [PR: 1.79 (95%CI: 1.43, 2.25)] (figure 3.5). None of the studies provided adjusted PR estimates.

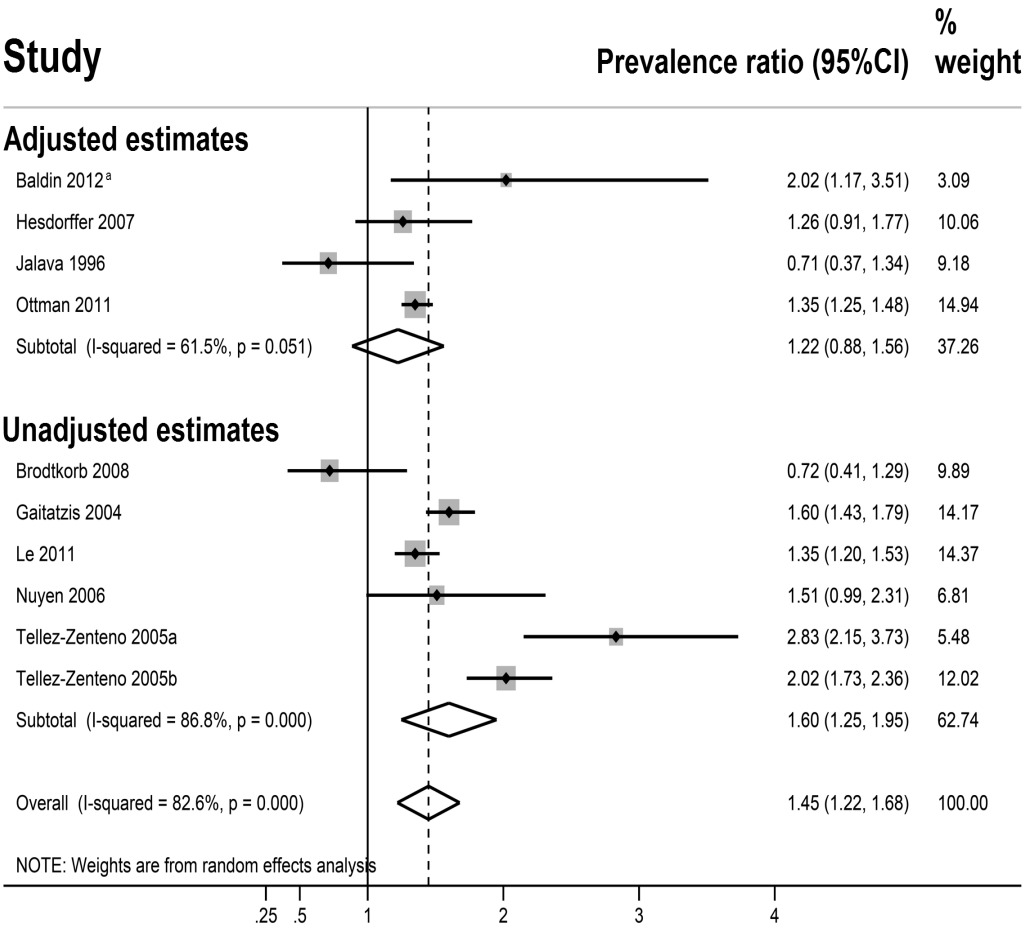

Figure 3.4: Prevalence ratio of migraine in people with epilepsy, stratified by adjustment for confounders. *[a]The unadjusted prevalence ratio (95%CI) reported by Baldin 2012 was 1.84 (1.18, 2.85).*

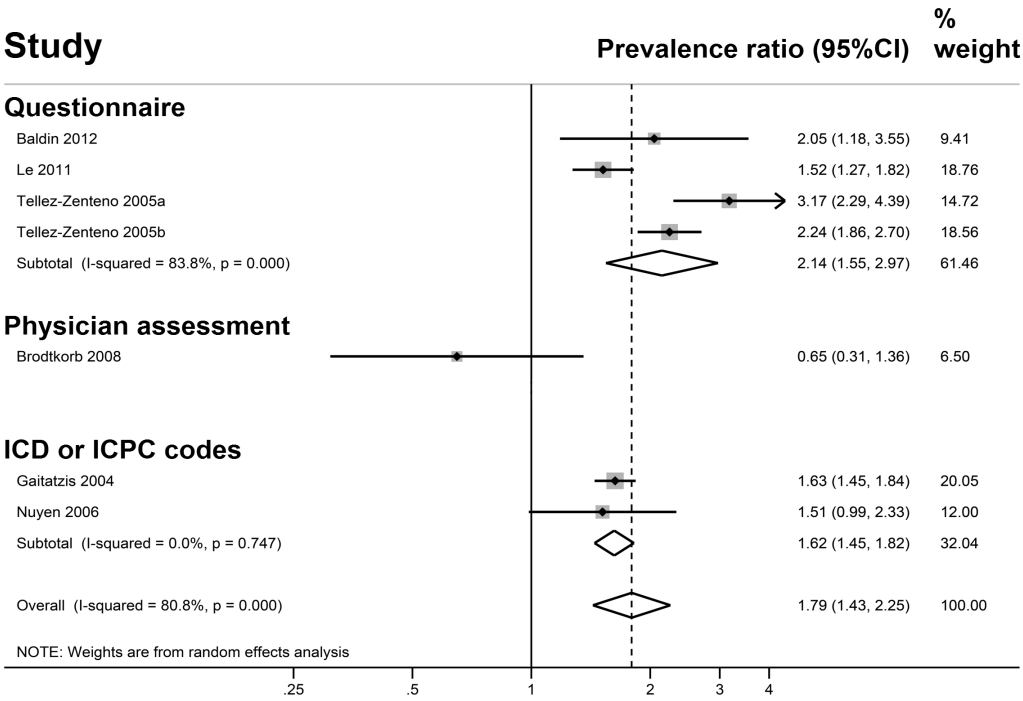

Figure 3.5: Prevalence ratio of epilepsy in people with migraine.

3.3.5 Publication bias

All five funnel plots were asymmetric upon visual inspection. This was consistent with our assumption that there was publication bias.

3.4 Discussion

This study showed that the reported lifetime prevalence of migraine among people with epilepsy ranged from 1.7% to 33.6%, representing an overall 52% increase relative to people without epilepsy. We also showed that the reported lifetime prevalence of epilepsy among migraineurs ranged from 0.7% to 2.3%, representing an overall 79% increase relative to people without migraine. The method of case ascertainment appears to have been an important source of heterogeneity, likely more so than adjustment for potential confounders.

Our finding that there is an important comorbid relationship between migraine and epilepsy on population level, adds to pathophysiological evidence for a link between the two conditions that was discussed in **chapter 2.1.** This is the first systematic review and meta-analysis to examine the shared co-prevalence of migraine and epilepsy. Our exhaustive literature search identified 10 studies that included a total of 1,548,967 subjects. This allowed us to produce more precise and, we expect, more accurate PR estimates than the individual primary studies, while also allowing us the opportunity to explore the reasons for any heterogeneity between studies. Another important aspect of our systematic review was that we chose to measure and report PRs, as opposed to PORs, given that the latter are notoriously difficult to interpret and have been consistently shown to over-estimate relative probabilities, especially when the dependent variable is common (Fisher *et al.*, 2005; Knol *et al.*, 2012; McNutt *et al.*, 2003). Finally, we limited study eligibility to population-based studies. The accuracy of case ascertainment is a greater challenge in population-based studies, where cases are generally identified using screening questionnaires (as discussed further below), than in hospital or clinic-based studies, where cases are generally identified by an expert physician using strict diagnostic criteria. That said, population-based studies reduce the risk of selection bias, as well as increase external validity/generalisability, which would otherwise result in over- or under- estimates of the true co-prevalence of migraine and epilepsy in the general population.

A potential source of bias in the identified studies was the irregular efforts to control for confounding. It remains unclear to what degree the association between migraine and

epilepsy is due to the shared effect of potential confounders (e.g. age and sex). We carried out a subgroup analysis comparing the adjusted to the unadjusted PR estimates where it seems that adjustment removed what had seemed to be a significant increase in the prevalence of migraine among people with epilepsy. That said, much of the adjusted pooled estimate was driven by one outlier study (Jalava and Sillanpää, 1996) which may have biased the pooled estimate towards the null. It is also worth noting that the adjusted estimate from Baldin *et al* was greater than the unadjusted estimate (Baldin *et al.*, 2012). Only one of the primary studies was primarily of children (Baldin *et al.*, 2012) and all studies were carried out in Western Europe or North America, potentially limiting the generalisability of our findings to other populations.

A potentially serious methodological issue we identified in most of the primary studies was the use of un-validated tools (which were generally in the form of questionnaires) to identify cases of migraine and/or epilepsy in the general population. Most studies also failed to specify whether they adhered to a particular operational definition of epilepsy or migraine such as those proposed by the International League Against Epilepsy (Berg *et al.*, 2010) and International Headache Society (Cianchetti *et al.*, 2013; Olesen *et al.*, 2013), threatening the external validity of their findings. The two studies that used validated questionnaires were still open to potential misclassification bias, given that both questionnaires were reported to have very high specificities but sensitivities of approximately 77%, meaning that 23 of every hundred cases of epilepsy or migraine went undetected.

It is important for physicians to be aware of the possible association between epilepsy and migraine. It was previously suggested that people with epilepsy with migraine are more likely to have a poor epilepsy prognosis as compared to the people with epilepsy without migraine (Velioglu *et al.*, 2005). The comorbid association between migraine and epilepsy has therapeutic implications as well. For example, certain AEDs can be used as migraine prophylaxis. Further studies are required to better understand the comorbid relationship between epilepsy and migraine. Special care should be taken to use accurate methods for the identification of cases of migraine and epilepsy, and to specifically distinguish between different temporal associations (e.g. interictal, preictal, ictal and postictal migraine) (Cianchetti *et al.*, 2013). Controlling for potential confounders, age and sex at the very least, should also be a priority. Further research should investigate the degree to which the relationship between migraine and epilepsy is influenced by factors such as age and the

presence of migrainous aura. If the association between migraine and epilepsy is due to a common genetic substrate, it would also be reasonable to expect that the link between migraine and epilepsy would be most evident among those with genetic forms of epilepsy.

4

Understanding the postictal state in epilepsy through computational modelling

"Catalysed by a hypothesis we may find something in the data that we were not looking for, but this is better than finding nothing at all - which is precisely what happens to the entirely passive observer of natural phenomena."[4]

4.1 Introduction: brain states in epilepsy

4.1.1 Brain states

One of the enigmatic features of paroxysmal neurological conditions, such as epilepsy and migraine, is the fact that sudden transitions occur between normal and pathological functioning of the brain. Often it is not clear what causes the transition between normal and pathological functioning. A proportion of people with epilepsy and migraine report clear triggers for their attacks, such as stress, flashing lights, the menstrual cycle, or sleep deprivation (Wassenaar *et al.*, 2014). We can think of the normal functioning of the brain and the pathological functioning as separate brain states. The term "brain state" is often used in the neuroscience literature but ill defined (Brown, 2006). Waking, sleeping, and dreaming are seen as separate brain states, but

[4] Ramón y Cajal S., (translation by Swanson N, Swanson LW). *Advice for a young investigator.* The MIT Press, Cambridge, MA, 1999, p117

other states such as coma and lucid dreaming have also been described as brain states (Brown, 2006). The differences between these states are obvious, especially when considering the *behaviour* associated with them. When delving deeper to understand the neural correlates of these states, things become more complicated, not in the last part because the question of consciousness arises. In waking, sleeping, coma, and dreaming the conscious experience is altered (or maybe even absent). The questions then arise: what exactly is consciousness, and why it is altered in these brain states? These extremely difficult questions can also be asked in the context of epilepsy, as consciousness is often affected during epileptic seizures. In the experiments I have done and will describe here, I did not address behaviour and consciousness. Throughout the experiments reported in this thesis I have taken a materialistic approach and only investigate the differences in brain functioning. A study of consciousness in this context would require a more philosophical approach and is a matter for future studies. From a purely materialistic, neurological point of view, it can be asked: what changes occur in the brain to cause the different behaviours such as sleeping, dreaming, and waking? Several hypotheses have been put forward, but so far none of these have been fully proven or fully refuted. It was proposed that neurotransmitters play an important role in changing and maintaining brain states (Brown, 2006; Veening and Barendregt, 2010; Yu *et al.*, 2015). Another hypothesis is that brain states are related to changes in the firing patterns of neuronal assemblies (Buzsáki *et al.*, 2013). Neurons can fire in unison, not only with their direct neighbours but also with (groups of) neurons that are more distant. This firing can be phase-locked, so that the neurons fire at the same time in the same frequency, even if they are spatially distant from each other. This synchrony "binds" spatially separated neuronal assemblies in time, providing the brain with an additional dimension: time (Buzsáki *et al.*, 2013). The neurotransmitter and synchrony hypotheses are not separate: neurotransmitters influence firing patterns of the brain and vice versa. Throughout this work, the following operational definition of a brain state will be used: *"groups of neurons firing at the same time in the same frequency"* (Brown, 2006).

4.1.2 Dynamics of brain states in epilepsy and migraine

In epilepsy and migraine, there is a pathological brain state in addition to the physiological brain states such as sleeping and waking. In migraine, attacks are accompanied by increased sensitivity to light and sound, and autonomous symptoms

such as nausea, vomiting, tiredness, and polyuria (Charles, 2013). The neural correlates of this pathological state are incompletely understood, but it has become clear that it involves changes in the connectivity between brain regions and altered firing patterns of neuronal assemblies (Charles, 2013). The pathological state in epilepsy and its neural correlates are more obvious. During epileptic seizures, neuronal assemblies exhibit "hypersynchrony", a state of excessive synchronous firing that can involve a part of the brain (focal seizure) or the whole brain (generalised seizure). This has led mathematicians and physicists to describe the epileptic brain as a "bi-stable" system with two equilibrium "attractor states"- a normal and a seizure state (figure 4.1). Perturbations that exceed a critical threshold can cause changes in the neuronal assemblies that lead to sudden transitions from one state to another. This is also called "non-linear behaviour", a term used to describe the relation between the strength of the perturbation (input) and the behaviour of the system (output). In a linear system, the output varies proportionally with the input, but in a non-linear system, there is a critical threshold. If the input is below this critical threshold, little changes in the output. When the input is higher than this critical threshold, there is a proportionally much larger change in the output (figure 4.1).

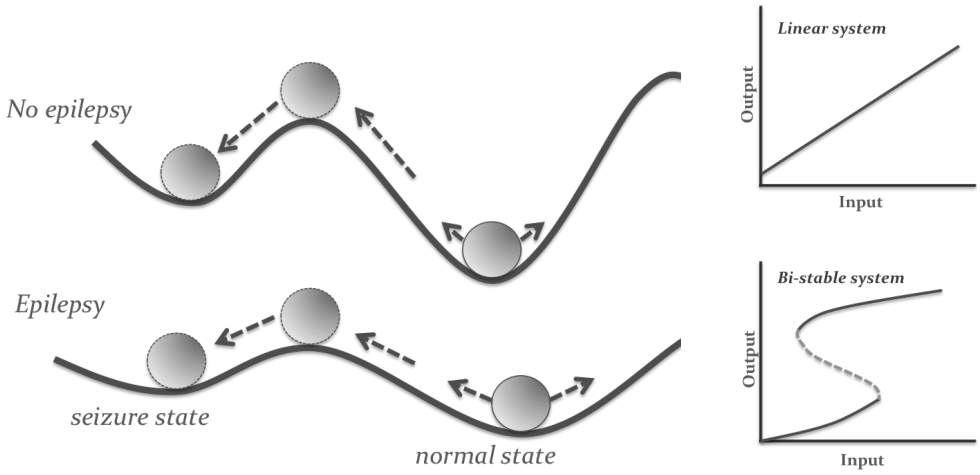

Figure 4.1: Bi-stability. *Top left: in people without epilepsy, transitions from a normal state to a seizure state can occur under extreme situations – the attractor (valley) of the normal state is deep and narrow, making it stable, and the "way out" of this state is steep, showing that it requires a strong perturbation to transition to the seizure state. In epilepsy (bottom left), the attractor of the normal state is shallower, and a small perturbation can cause a transition to the seizure state. The right panel shows input-output curves of a linear system (top) and of a bi-stable, i.e. epileptic system (bottom).*

Transitions between epileptic states can thus be described as non-linear and bi-stable. This is a very simplified view of reality, but this abstraction into different states

enables the creation of abstract computational models that exhibit the same kind of behaviour. These models can help understand how the transitions between the physiological and pathological states in epilepsy occur. In the following sections I will describe a study in which my co-supervisors and I extended such a computational model to include the postictal state as a third distinct brain state. I describe how simulations of state transitions in this model of epilepsy provide hypotheses that can be tested in human EEG recordings.

4.1.3 Deriving hypotheses from computational models

Knowledge about seizure initiation or the transition from normal to ictal states is increasing, but less is known about seizure termination. Most generalised tonic clonic seizures lead to a postictal state that is clinically and electrographically distinct from the ictal and interictal states. In the Electroencephalography (EEG) this manifests as slowing, or as total suppression of the background activity, termed Postictal Generalised EEG suppression (PGES, see figure 4.2) (Lhatoo *et al.*, 2010; So and Blume, 2010; Surges *et al.*, 2011). During a PGES event, people are mostly immobile and in an unresponsive, coma-like state (Semmelroch *et al.*, 2012; Seyal *et al.*, 2013; Tao *et al.*, 2013). This event is thought to be an extreme expression of the postictal state. It is clinically relevant as these events consistently preceded cardiorespiratory arrest in most reported ictal recordings of Sudden Unexpected Death in Epilepsy (SUDEP) (Ryvlin *et al.*, 2013). PGES also frequently follows non-fatal convulsive seizures. Whether PGES is also a risk factor for SUDEP is a matter of debate (Lamberts, Gaitatzis, *et al.*, 2013; Lhatoo *et al.*, 2010; Surges *et al.*, 2011).

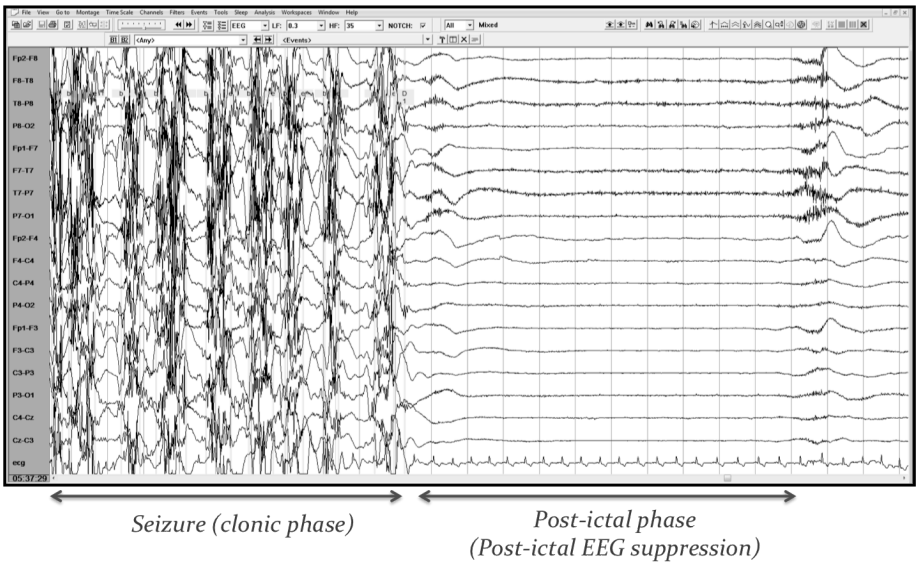

Figure 4.2: EEG recording with postictal generalised EEG suppression. *Low pass filter 0.3Hz, high pass filter 35Hz.*

Seizure termination may occur either due to a random process involving external perturbations or fluctuating state parameters, or due to a deterministic, autonomous neuronal mechanism driven by the ictal condition itself (Kalitzin *et al.*, 2010; Kramer *et al.*, 2012; Lopes da Silva *et al.*, 2003a, 2003b; Stamoulis *et al.*, 2013). Our objective is to clarify the type of dynamics underlying termination of convulsive seizure and the subsequent postictal state. We developed a computational neural mass model that autonomously transitioned between seizures and normal states. With the findings from this model we attempt to understand features of state transition in EEG recordings of human convulsive seizures.

Computational models of seizures, based on neuronal lumps, have previously been used to describe global dynamics of state transitions. Seizure transitions are thought to occur in bi-stable systems where a stable "attractor state" corresponds to normal activity and a second, transient quasi-stable "limit-cycle state" represents seizures. Probability distribution statistics, particularly the gamma distribution, can be used to distinguish between stochastic and deterministic processes. Seizure onset of some types of seizure was shown to have properties of a random walk-type stochastic process, while seizure termination may be influenced or even governed by deterministic processes (Koppert *et al.*, 2011). This was consistent with experimental and clinical data (Colic *et al.*, 2013; Suffczynski *et al.*, 2006). In these studies, postictal states were not considered. In this study, we extended these findings to account for seizure termination and the postictal period. The computational model presented here is an extension of a model of multiple bi-stable units (Koppert *et al.*, 2014), with added activity-driven connectivity dynamics. This model displays transitions from ictal to postictal and from postictal back to normal states. Critically, I tested and validated the hypotheses derived from this computational model against EEG recordings of convulsive seizures from 48 people with refractory epilepsy. A better understanding of the dynamics of seizure termination may help the development of new approaches to prevent the severe complications associated with PGES.

4.2 Methods

4.2.1 Introduction of the computational model

Computer simulations were carried out using a simplified lumped neuronal mass model created in Matlab® (release 2014b, The MathWorks Inc., Natick, MA, USA). The

purpose of this abstract model is to explain the general *dynamics* of state changes in neuronal populations including pyramidal cells and interneurons while preserving essential properties of realistic neuronal networks (Kalitzin *et al.*, 2014; Koppert *et al.*, 2014). The model consists of 128 fully interconnected units, with equal connectivity between any two units (Figure 4.3). Each *single unit* is a simple system that can have two dynamic states, depending on the chosen parameters. The first is a harmonic oscillator representing the normal, non-excited state of a neuronal mass. The second is a limit cycle attractor with permanent stable oscillations representing a micro-seizure (Izhikevich, 2001; Kalitzin *et al.*, 2010). For certain parameter ranges both states co-exist (bi-stability), for other parameters values the unit is in one of the states. In the bi-stable regime the transitions between the two states can be induced by external inputs or by random fluctuations. In this study we use an analytical model that provides bi-stability in a relatively simple way. The model represents the collective dynamics of multiple pairs of excitatory and inhibitory populations, each represented by a complex variable $Z_m, m = 1..M$. These degrees of freedom incorporate the excitatory population dynamics and the inhibitory one as real and imaginary components correspondingly ($Z_m(t) = Exc_m(t) + iInh_m(t)$). The original definition of the model (Koppert et al 2014) is:

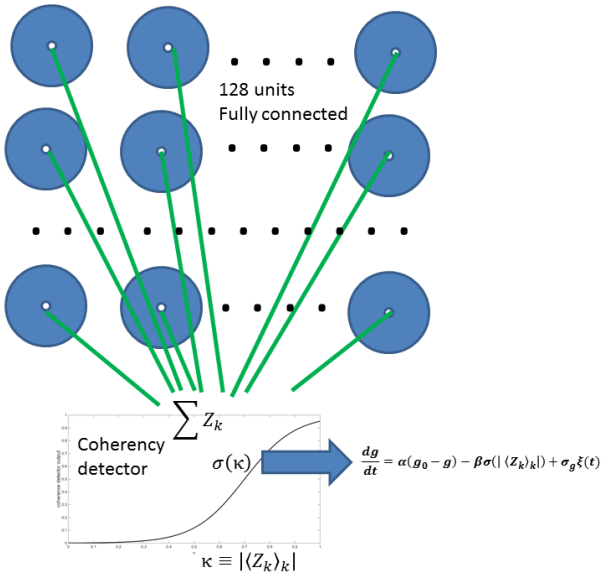

Figure 4.3: Schematic representation of the computational model*. The model consists of 128 fully interconnected units, representing neuronal lumps including pyramidal neurons and interneurons. Any two units are equally interconnected. The collective output of all units is filtered through a sigmoid function or coherency detector (input-output function in inset and equation (2)). The horizontal axis represents the collective output of the model, and the vertical axis is the detector response. The output of the coherency detector is used as input for the dynamics of the connectivity parameter g, which is common for all units.*

$$\frac{d}{dt}Z_m = -|Z_m|^4 Z_m + b|Z_m|^2 Z_m + cZ_m \; + i\omega Z_m + g(1+i)\sum_{k=1}^{N} C_{mk} Z_k + \sigma_z \eta(t) \quad (1)$$

In the above equation, b, c and ω are parameters of the single unit dynamics, the matrix C_{mk} represents the interactions between the units k and m, N is the total number of units in the network; g is a connectivity scaling coefficient, and $\eta(t)$ is a random complex variable with normal distribution of unit variance; $\eta(t)$ and the scaling coefficient σ_z introduce noise in the system. The factor (1+i) reflects complex interactions between inhibitory and excitatory subunits in the system. The overall layout of the network and schematic flow of interactions is shown in figure 4.3. Parameters c and b represent the global balance between excitation and inhibition within a single oscillatory unit (Koppert *et al.*, 2014). Depending on these parameters, the unit can be in a steady state, a limit cycle, or both (bi-stability).

We selected the parameters ($c = -2.26, b = 3, \omega = 0$) such that each individual unit is in one state – that of a fixed point harmonic oscillator (normal, non-seizure state). The behaviour of the connected system is therefore a collective emergent property, influenced by the connectivity strength determined in parameter g. We carried out two series of model simulations. First, a series of simulations for an array of units with different levels of connectivity (range [0,1/128], 101 values) was done under stationary parameters without external input or noisy perturbations. The purpose of these "stationary state" simulations was to explore the diversity of asymptotic states of the model, depending on the connectivity parameter g and the initial conditions. For each connectivity value, 129 simulations were performed with increasing numbers of units (from 0 to 128) in an activated state of limit cycle as initial condition. The connectivity matrix for all simulations in this study was chosen arbitrarily $C_{mk} = 1, m \neq k, C_{mm} = 4$ to represent the relative difference in local versus global connectivity.

The second series of simulations was performed to obtain dynamical seizure transitions and postictal states. Noise was added to the system, and a parameter evolution rule was introduced, consisting of negative feedback plasticity that drives the connectivity parameter g to lower values whenever the global synchronised activity of the system exceeds a threshold (see equation (2)). In addition, homeostatic point stochastic dynamics were introduced for the parameters b and c to account for random-walk type of fluctuations of the operational point of the model.

$$\frac{dg}{dt} = \alpha(g_0 - g) - \beta\sigma(|\langle Z_k\rangle_k|) + \sigma_g\xi(t)$$
$$\frac{dc}{dt} = \alpha(c_0 - c) + \sigma_c\mu(t) \ ;$$
$$\frac{db}{dt} = \alpha(b_0 - b) + \sigma_b\nu(t) \ ;$$
$$\sigma(x) \equiv e^{\frac{x-x_0}{s}} \Big/ (1 + e^{\frac{x-x_0}{s}})$$
(2)

In equation (2) α_g and β are rate constants that determine the relaxation of the g-parameter and its corresponding reaction to increased coherency between the units. α_c and α_b are rate constants for the fluctuating $\{c,b\}$-parameters to a fixed homeostatic point $\{c_0,b_0\} = \{-2.26, 3\}$. The second term in equation (2) is a shortened version of an external unit, that according to previous results, can be activated by the network when the phase coherency of the system exceeds a certain level (Koppert *et al.*, 2014). The last terms $\xi(t), \mu(t), \nu(t)$ in equation (2) represent noise and are independent random variables with normal distributions of unit variance. To reduce the complexity of the model we emulated the activation process by an effective sigmoid function, as defined by the last line of equation (2). We performed 100 stimulations, initialising the system with all units having positive real values of > 1. This started the simulation with the system in a limit cycle with all units recruited, i.e. a "seizure". We recorded the time (number of simulation steps) it took for the limit cycle ("seizure") to be destroyed by the change in connectivity g and the time it took to return to a level of excitability in the "normal" range, which we defined as an excitability threshold 50% higher than that of the homeostatic point. This was used as an estimate of the duration of the model postictal period reflecting PGES. For this second set of stimulations we chose $\{\alpha_g, \alpha_c, \alpha_b\} = 0.002$, $\beta = 0.007$ $g_0 = 0.0045, x_0 = 0.1$ and s=0.05, which provided a single homeostatic point. The simulations were done with noise levels of $\sigma_z = 3, \sigma_c = 2, \sigma_b = 2, \sigma_g = 0.02$.

4.2.2 Statistics of state durations

It has previously been shown that differences in the distributions of durations between stable and transient states can be revealed using a gamma-type probability density function as a fitting template (see equation (3)) (Colic *et al.*, 2013; Suffczynski *et al.*, 2006).

$$\mathrm{N}(T) = N_0 T^{\alpha-1} e^{-T/\beta} \qquad (3)$$

Where N_0 is the normalisation constant, β is the time-decay constant, and α is the shape parameter, which separates random from deterministic processes. In short, α ≤1 is expected for the distribution of stochastic processes (Poisson process), while α >1 describes a deterministic process, and a normal distribution if α approaches 10 (Doob, 1953; Suffczynski *et al.*, 2006). In order to study the dynamics underlying the transitions in our model, we simulated 110 "seizures" followed by 110 model "postictal" periods. The segmentation of these epochs was performed using the thresholds of the envelope of the averaged signal from the unit output.

4.2.3 Human EEG data

We screened the video-EEG reports of people aged >15 years who underwent pre-surgical evaluation at Stichting Epilepsie Instellingen Nederland (SEIN) and selected those that mentioned the recording of a convulsive seizure. Only the first convulsive seizure recorded from each individual was selected to avoid effects of seizure clusters. For most, AEDs were tapered during the recording to maximise the likelihood of an ictal recording. In view of the changes to AED regimens, we chose not to include periods between two seizures. In total, 56 convulsive seizures were identified. One recording was excluded due to insufficient postictal recording time, and two due to inadequate EEG quality. From the 53 remaining recordings, convulsive seizures with an asymmetric partial ending (unilateral clonic movements and/or partial epileptic activity, four seizures), or convulsive seizures ending with generalised activity without convulsive movements (one seizure) were excluded, leaving 48 seizures. The subject characteristics are shown in table 4.1.

Data from this database were published previously (Lamberts, Gaitatzis, *et al.*, 2013; Lamberts, Laranjo, *et al.*, 2013). The scalp EEG recordings used the international 10%-20% system at a sampling rate of 200Hz (Stellate Harmonie, Stellate Systems, Montreal, QC, Canada). Two experienced clinical neurophysiologists (Roland Thijs and Dimitri Velis) independently marked the start of the seizure, the tonic phase, the clonic phase, the end of the seizure, and the start and end of PGES periods. These were defined as periods immediately postictal (within 30s), with generalised absence of electroencephalographic activity >10uV in amplitude, allowing for muscle, movement, breathing and electrode artefacts (Lhatoo *et al.*, 2010). All PGES periods longer than 1s were scored (Surges *et al.*, 2011).

Table 4.1: Patient characteristics.

	PGES+ N=37	**PGES-** N=11	**statistical test**
gender			
Male (N)	20 (54%)	8 (73%)	F, p=0.319
age at time of EEG			MW, p=0.081
year (median; range)	36.1 (15-61)	28.3 (16-43)	
Duration of epilepsy			MW, p=0.581
year (median; range)	18.4 (2-46)	21.3 (4-42)	
Epilepsy classification			
Symptomatic (N)	27 (70%)	11 (100%)	F, p=0.089
Unknown/genetic (N)	10 (30%)	0 (0%)	
Ictal EEG onset (%)			
Temporal (N)	18 (49%)	5 (45%)	F, p=1.00
Extra-temporal (N)	19 (51%)	6 (55%)	
Frequency of CS			
1-2 CS/year (N)	20 (54%)	5 (45%)	F, p=0.736
>3 CS/year (N)	17 (46%)	6 (55%)	
Total duration of seizure			MW, p=0.573
sec (median;range)	122.3 (63-444)	221.2 (45-828)	
Duration of TC phase			MW, p=0.202
sec (median;range)	66.01 (32-118)	73.8 (36-100)	
Duration of PGES			
sec (median;range)	55.7 (2-252)	NA	

PGES = postictal generalised EEG suppression, F= Fisher's exact test, MW= Mann-Whitney U test, CS= convulsive seizure, TC= tonic-clonic

In the EEG, I marked the beginning and end of every epileptic discharge, and corresponding artefact of the clonic movement, which I verified by video (I refer to this as a "clonic discharge"). I was blinded for the presence of PGES and only assessed the ictal EEG. For the visual inspection of the EEG signal I used a 0.3Hz low-pass and 35Hz high-pass filter. The sensitivity was 5-7.5uV and I used a longitudinal bipolar montage ("double banana"). I then imported the time of the markers I set in the EEG into Matlab® (release 2014b, The MathWorks Inc., Natick, MA, USA) and calculated the difference between the onset of each two adjacent "clonic discharges" in ms.

4.2.4 EEG analysis

The change in clonic frequency in the EEG was quantified by fitting a linear equation to the logarithm of the interclonic interval. If the times of successive clonic discharges

for a given seizure are t_k (marked by visual inspection of the EEG traces), then exponential slowing down can be formulated as (equation 4a).

$$ICI_k \equiv t_{k+1} - t_k = C_0 e^{a\,\tau_k}; \tau_k \equiv {}^{(t_{k+1} + t_k)}/_2 \qquad (4a)$$

The linear fit between the logarithm of the interclonic interval, and the middle time of the interval between each two successive clinic discharges τ_k provides the quantity that characterises the decrease of the rate of "clonic discharges".

$$\log(ICI) \approx a\tau + \log(C_0) + \varepsilon \qquad (4b)$$

In equation (4b) the fitting parameters and $\log(C_0)$ are obtained using the standard MatLab fitting routine *polyfit* applied to linear order (n=1). The last term in (4b) is a random variable representing the deviation from the fit. Its variation $r = var(\varepsilon)$ is the residual variance after the fit. The residual variance was used to estimate the "goodness of fit" of the logarithmic fit. From (4b) it follows that:

$$var(\log(ICI)) \approx \frac{var(ICI)}{ICI} = var(\varepsilon) = r;\ GOF \equiv 100(1 - r); \quad (5)$$

The total effect of ictal slowing for each seizure is quantified as:

$$ICI_{term} \equiv C_0 e^{a\,T_{seizure}} \qquad (6)$$

In the above definition the C_0 and a parameters are derived for each case from the linear fit procedure in equation (4b), and $T_{seizure}$ is the total duration of the seizure. The actual values of the first and last interclonic interval measured experimentally are influenced by noisy perturbations. We therefore use the projected terminal interclonic interval values assuming that the noisy component, ε, in equation (4b) has been largely filtered out by the fitting procedure. We call the quantity defined in equation (6) projected terminal interclonic interval ($ICI_{terminal}$).

To test whether the PGES durations (set to zero if no PGES is detected) $\{T_{PGES}\}$ and the corresponding $\{ICI_{term}\}$ of the convulsive seizure are functionally related, we used the unidirectional h^2 nonlinear association measure (Kalitzin *et al.*, 2007). The association

index estimates the variance of one time series, x, which can be explained by the variance of a second time series, y, and in this way quantifies the exactness of the best functional map between the two time series.

$$h^2(x,y) = 1 - \frac{var(x|y)}{var(y)} \qquad (7)$$

The unidirectional nature of the index in equation (6), (or the non-symmetric relation $h^2(x,y) \neq h^2(y,x)$, reflects the fact that not all functions are invertible. A surrogate-based test that establishes the statistical significance of the h^2 index was derived (i.e. estimates the probability of obtaining the given association index by chance). In the present study we chose the number of bins, the only instrumental parameter needed, as 10. For the statistical significance validation of the associative index, we applied 100,000 surrogate tests. The distributions of the $ICI_{terminal}$ quantities, ρ from equation (6), and the goodness of fit values from equation (5), as functions of the PGES duration were estimated. The set $\{T_{PGES}\}$ was divided into bins with unequally spaced borders at [0 10 50 100 200 500] seconds. Significant differences were detected using the non-parametric Kruskall-Wallis test.

4.3 Results

4.3.1 Characteristics of the neuronal mass model under stationary parameters

To elucidate the type of dynamics underlying seizure termination and PGES, we created a computational model, which we first analysed under stationary parameters. The *entire system* has three different dynamic regimes depending on the connectivity g, shown in figure 4.4 (Koppert *et al.*, 2014). For lower values of g the system is not excitable. This state represents PGES as an extreme of the postictal state (blue region on the left in figure 4.4). For higher values of g, the system is in a non-excitable, stable state depending on the initial conditions or external perturbations. This represents normal brain functioning. Finally, when g is large, the system has only one asymptotically stable state (attractor), which is a limit cycle of all units oscillating synchronously, representing an epileptic seizure. We reproduced the above model in the interaction term as previously with only the real components of the units (Koppert

et al., 2014). Each individual unit has one state (*embedded properties)*, while the *system* of connected units can have different states. We identify these states and the transitions between them as *emergent properties* of the model.

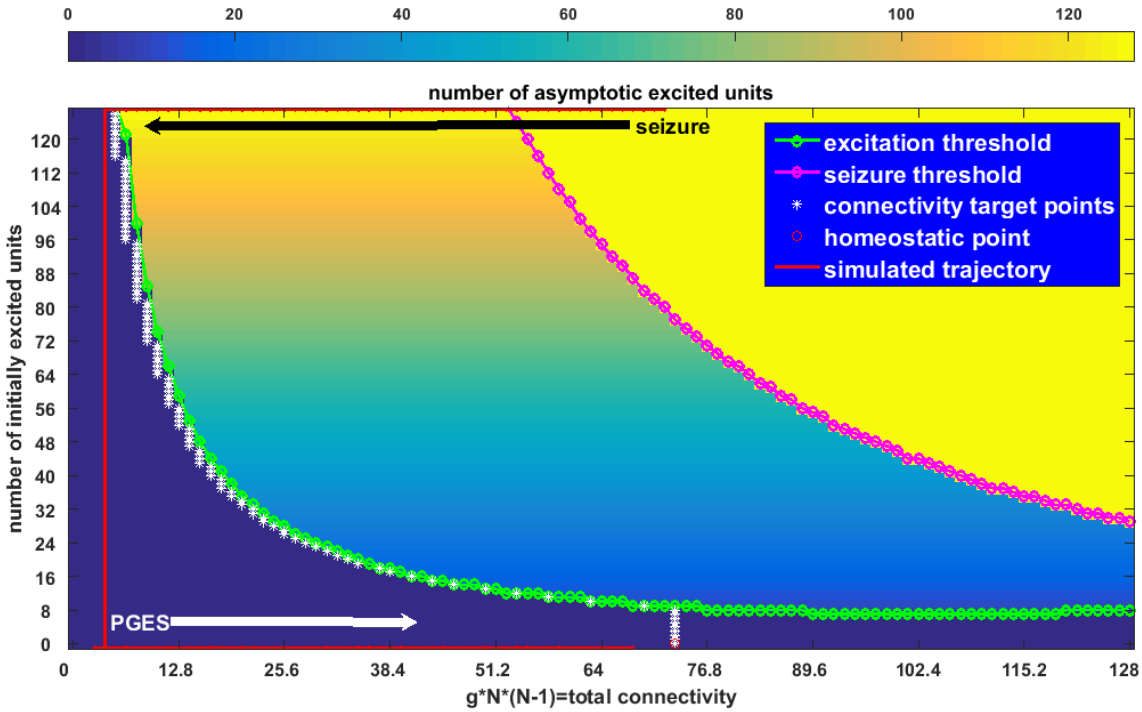

Figure 4.4: Output from the computational model. *Results from simulations of the system (equation (1)). The system output is generated for 129 values of the connectivity parameter g, ranging from 0 to 128 on the horizontal axis, and for 0 to 128 initially excited units, indicated on the vertical axes. The background colour represents the number of excited units that remain self-sustained according to the dynamics of the coupled system of oscillators. All simulations were first done without noisy input and without changes of the connectivity parameter g. The blue region corresponds to a non-excitable state ("postictal"); yellow to a limit cycle state (total synchronization or "seizure"); and the gradually coloured state in the middle, to "normal functioning", where the system sustains its initial state.*

Introduction of noise and plasticity of connectivity g through the coherency detector (equation (2)), makes the system transition between the different states (red line). The model simulation starts in a "seizure" state. The connectivity parameter g is activated above a certain level of synchrony (the input from the coherency detector from fig. 1). This "seizure"-induced plasticity of the connectivity parameter g causes termination of the "seizure" and drives the return through a "postictal" period to the "normal" state, defined as an excitability threshold 50% higher than that of the homeostatic point, indicated in red.

4.3.2 Characteristics of the neuronal mass model with activity-dependent plastic feedback parameter dynamics

To make the model transition autonomously between seizures, postictal periods and normal periods, we introduced random noise and a negative feedback plasticity rule, which drives the connectivity *g* to smaller values whenever the global synchronised activity of the system exceeds a certain threshold (equation (2)). When random fluctuations bring the system above the "recruitment threshold", the system enters full synchrony, or a "seizure" state. Figure 4.4 shows a simulated trajectory (red line with arrows) as an example of a succession of these dynamic states: from the seizure state, to the non-excitable postictal state, and back to the normal state. The transition to the postictal state is determined by the influence of the connectivity change (equation (2)) causing a transition to a temporary state of low inter-unit connectivity, with low values of the connectivity parameter *g*, where the system is silent and non-excitable. The connectivity then gradually increases again until the system is in its normal state. The system stays in its homeostatic domain ("normal operation") most of the time, but it can make a transition to a fully synchronised state ("seizure") because of external input or random noise fluctuations exceeding the recruitment threshold.

4.3.3 Predictions about seizure termination according to the neuronal mass model

Our computational model has three essential features that were used to analyse the human EEG data of convulsive seizures. First, the shapes of the distributions and the parameter values suggest that the duration of the "ictal" (α=237,6 95%CI [180.1 - 313.5]) and "PGES" (α=21.2 95%CI [16.1 – 27.9]) epochs have deterministic properties. In figure 4.5, the distributions of the epoch lengths and their corresponding gamma-distribution fits are shown. This leads to the *first hypothesis (H1*): the durations of the convulsive seizure and PGES events in humans display distributions corresponding to deterministic termination processes.

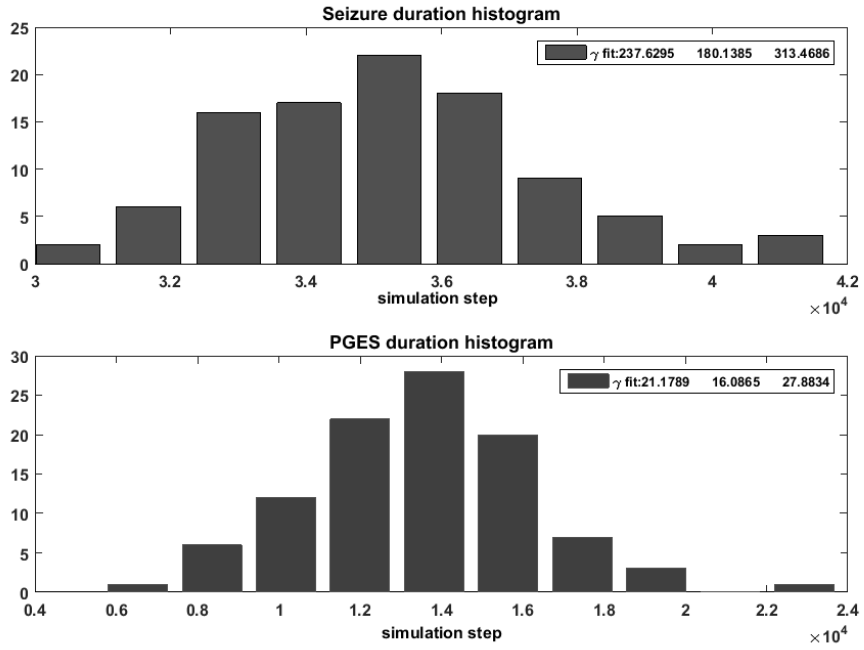

Figure 4.5: Gamma distributions of ictal and postictal period durations in the model. *Histograms and fitted gamma functions for the distributions of the "seizure" (top frame) and "postictal" (bottom frame) durations as simulated using the model. The estimation of the shape parameter α (from equation (3)), for the fitted gamma-distribution as well as the 95% confidence intervals (CI) are presented in the text boxes. The data were obtained using the standard MatLab® function gamfit.*

The second feature of the model is that seizure termination is influenced by the connectivity parameter g. This suggests the existence of a measurable quantity, reflecting changes in connectivity, which changes during a seizure until its termination. In our model, we coupled the evolution of connectivity parameter g to the global level of synchronisation of the system as expressed in the first line of equations (2), enabling the measurement of the interval between modelled clonic bursts or interclonic interval. Figure 4.6 shows that the interclonic interval increases as a function of the time elapsed from the start of the seizure. The connectivity changes exponentially as the seizure progresses, and the terminal value of the interclonic interval correlates strongly with the duration of the "PGES" state in the model (figure 4.6, bottom frame, h^2=0.82).

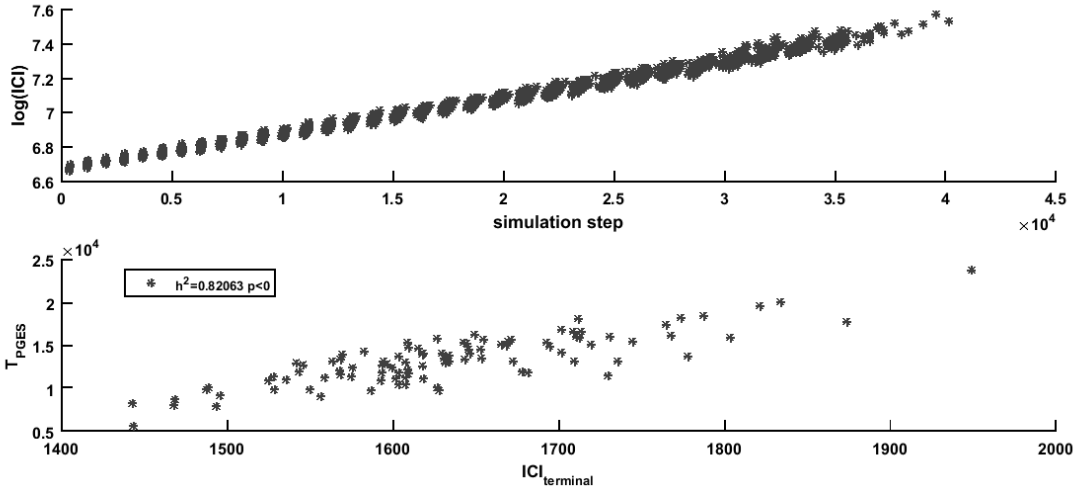

Figure 4.6: Relation between the interclonic interval (ICI) and PGES duration in the model. *Top frame: Scatter plot showing the relation between the interclonic interval (ICI, vertical axis, logarithmic scale) determined by the strength of the connectivity parameter g during simulated seizures and the time elapsed since the beginning of the simulated seizure (horizontal axis, in simulation steps). The different data points at each time point represent different simulations. The figure shows that the ICI is relatively constant at the start of the model seizure, but varies at the end of the seizure. Bottom frame: The relation between the terminal model ICI value and the duration of the PGES state in the model. The non-linear correlation coefficient h^2 shows that the terminal ICI value explains 82% of the variability of the PGES duration.*

Figure 4.6 shows the relation between connectivity parameter *g* and PGES duration, and between the terminal interclonic interval and the terminal value of *g* (figure 4.7). It was previously observed that the interclonic interval increases almost exponentially towards the end of a convulsive seizure (Beniczky *et al.*, 2014; Conradsen *et al.*, 2013). Our *second hypothesis (H2)* derived from the model and from clinical observations is, therefore, that the exponential change of the interclonic interval reflects the decrease of the connectivity *g* facilitating the termination of the seizure.

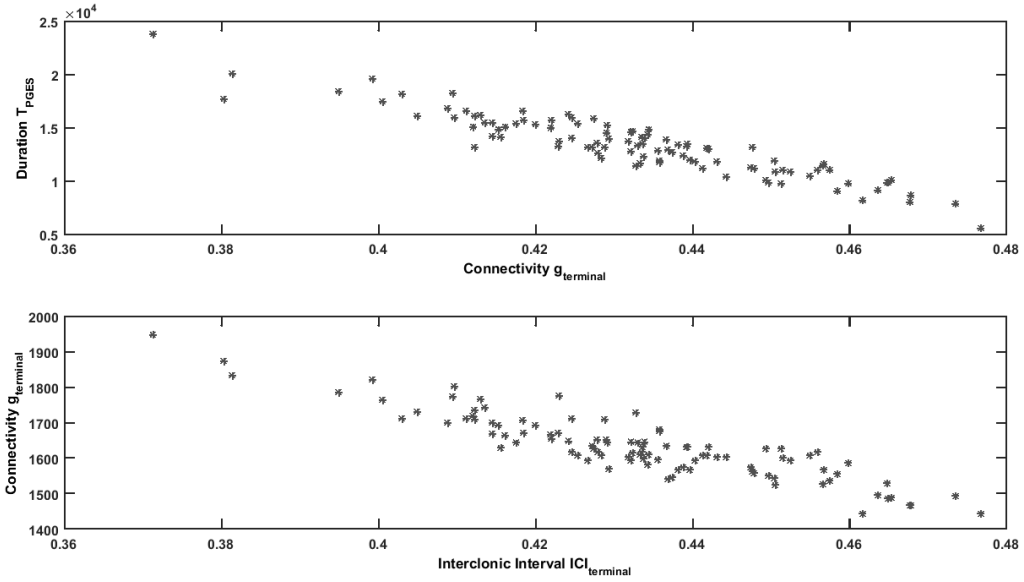

Figure 4.7: Relation between the connectivity, interclonic interval and PGES in the model. *Top frame: Scatter plot showing the relation between the durations of the simulated PGES states (vertical axis, in simulation steps) and the value of the connectivity parameter g at the end of the preceding seizure (horizontal axis, dimensionless units). Bottom frame: the relationship between the terminal value of the connectivity parameter g and the terminal interclonic interval in the model.*

Lastly, our third, and most important hypothesis *(H3)* is that the interclonic interval at the end of a convulsive seizure is associated with the duration of the following PGES period. This is motivated by the observation in our model that the dynamics of the connectivity parameter *g* during a seizure are involved in seizure termination and lead to a "PGES" state of suppressed activity (figure 4.4). The duration of this period is determined by the time needed for the connectivity parameter *g* to re-enter the normal operational state. In the absence of noisy input, this time depends on the value of the connectivity parameter *g* when the seizure terminates. Accordingly, the model shows that the duration of the postictal period is related to the connectivity parameter at the end of the seizure, reflected by the oscillatory frequency of the model, which, we hypothesise in *(H2),* corresponds to the interclonic interval. In the next section, these three features deduced from our neuronal model are tested in human EEG recordings of convulsive seizures.

4.3.4 Gamma distribution of human seizure and PGES durations

Of the 48 convulsive seizures, 37 ended with PGES (see table 4.1). Analogous to the model data, the duration of the seizures and PGES periods was assessed. The distribution of the durations and corresponding gamma-distribution fits are shown in figure 4.8. The seizure duration varied from 45 to 828s, and PGES periods lasted from 2 to 252s. The distribution of the durations of PGES (α=1.537 [95% CI 1.014-2.32]) in human EEG is indicative of a deterministic process. We confirm previous observations of a deterministic process, which probably underlies convulsive seizure duration (α=2.660 [95% CI 1.823-3.880]. Both findings are in line with (*H1)* from the model.

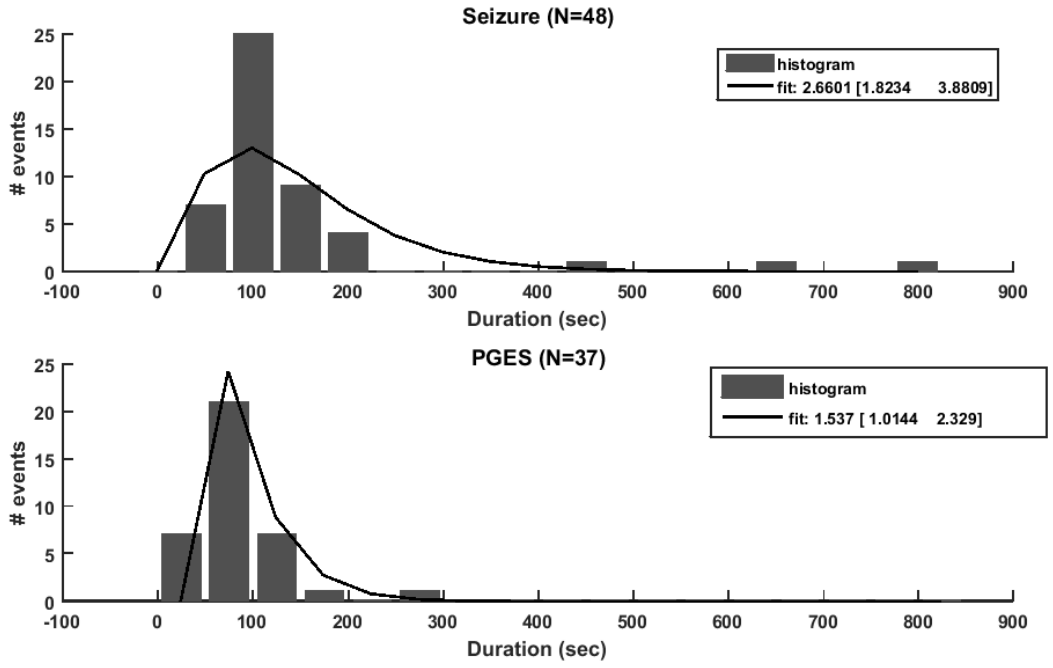

Figure 4.8: Gamma distribution of ictal and PGES period durations in human EEG data. *Histograms and fitted gamma functions (solid lines) for the distributions of the seizure (top frame) and PGES (bottom frame) durations as visually detected from the human EEG recordings. The three numbers in the legends give the parameter α (from equation (3)) for the fitted gamma-distribution and the corresponding 95% confidence interval as obtained from the standard MatLab® function gamfit.*

4.3.5 Clonic slowing at the end of a convulsive seizure follows an exponential pattern

The convulsive seizures in my sample ended with a clonic frequency between 0.5 and 1.5Hz, as estimated by visual inspection of EEG traces of epileptic discharges, and video recordings of corresponding clonic movements ("clonic discharge"). The clonic frequency decreased exponentially in most seizures. Examples of the linear fit of the logarithm of the interclonic interval from single seizures, as a function of time from clonic phase start, are shown in figure 4.9. The averaged goodness of fit for all 48 convulsive seizures was 73%, with standard deviation 14%. This confirms (*H2)* and previous observations (Beniczky *et al.*, 2014; Conradsen *et al.*, 2013).

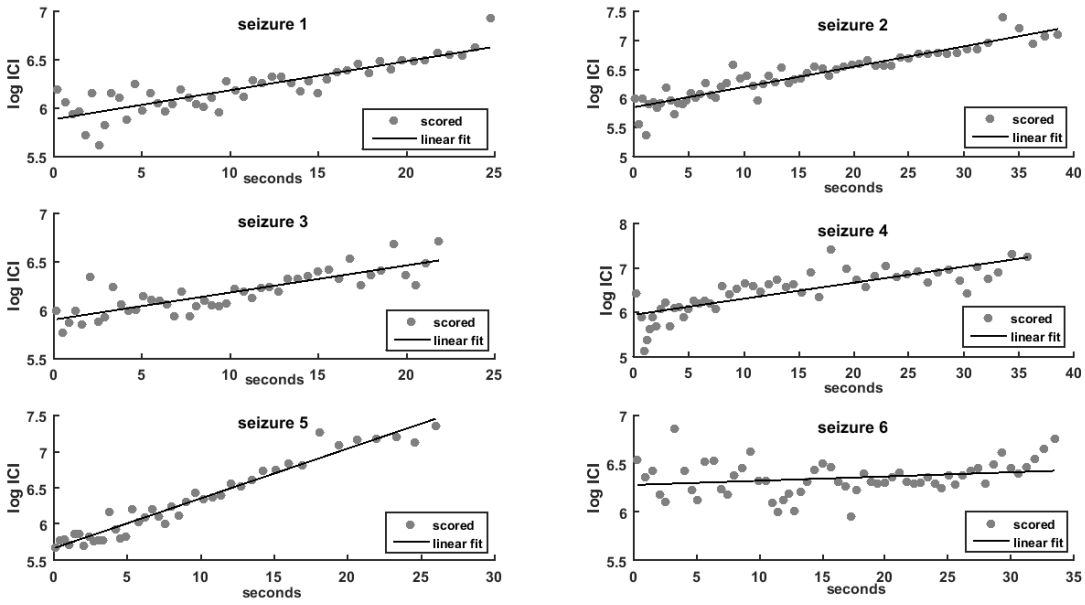

Figure 4.9: Linear fit of the interclonic interval (ICI) in human seizures*. Scatter plots of interclonic intervals (circles) and best linear fit (solid line) between the time from the beginning of the convulsive phase (in seconds, horizontal axis) and the logarithm of the interclonic intervals (log(ICI), vertical axis). The figure illustrates six seizures from the dataset, the fitting algorithm was applied to all 48 cases.*

4.3.6 Clonic slowing is associated with PGES duration

The logarithmic fit of clonic slowing was used to estimate the terminal value of the connectivity parameter at the end of real seizures, in analogy with the model. This value (projected terminal interclonic interval, $ICI_{terminal}$) was then correlated with the occurrence and length of PGES. A scatter plot depicting the $ICI_{terminal}$ and PGES lengths

is shown in figure 4.10. If there was no PGES the value of PGES was set to zero. $ICI_{terminal}$ explained 41% of the variance in PGES duration: h^2=0.41, p<0.02. PGES duration explained 34% of the variance in $ICI_{terminal}$: h^2=0.34, p<0.01 (figure 4.10). This is in keeping with (*H3)*, that the $ICI_{terminal,}$ possibly reflecting the decrease in connectivity, is correlated with PGES occurrence and duration. The larger the total deceleration effect, the longer PGES lasts. Several seizures in my sample, with a marked interclonic interval increase, however, did not end with PGES, but there were no seizures without an interclonic interval increase that ended with PGES. This makes clonic slowing a highly sensitive predictor of PGES in our data sample. The strongest association is seen between clonic slowing leading to a long $ICI_{terminal}$ and long PGES. The goodness of fit increased when seizure termination was followed by a longer PGES period. This corroborates with (*H3)*, i.e. that deterministic dynamics, typical of long $ICI_{terminal}$, also determine the presence and duration of PGES. In line with previous studies (Lhatoo *et al.*, 2010; Semmelroch *et al.*, 2012; Seyal *et al.*, 2012; Surges *et al.*, 2011), there was no correlation between the duration of the seizure and the $ICI_{terminal}$ in my sample (h^2=0.15, p<0.46).

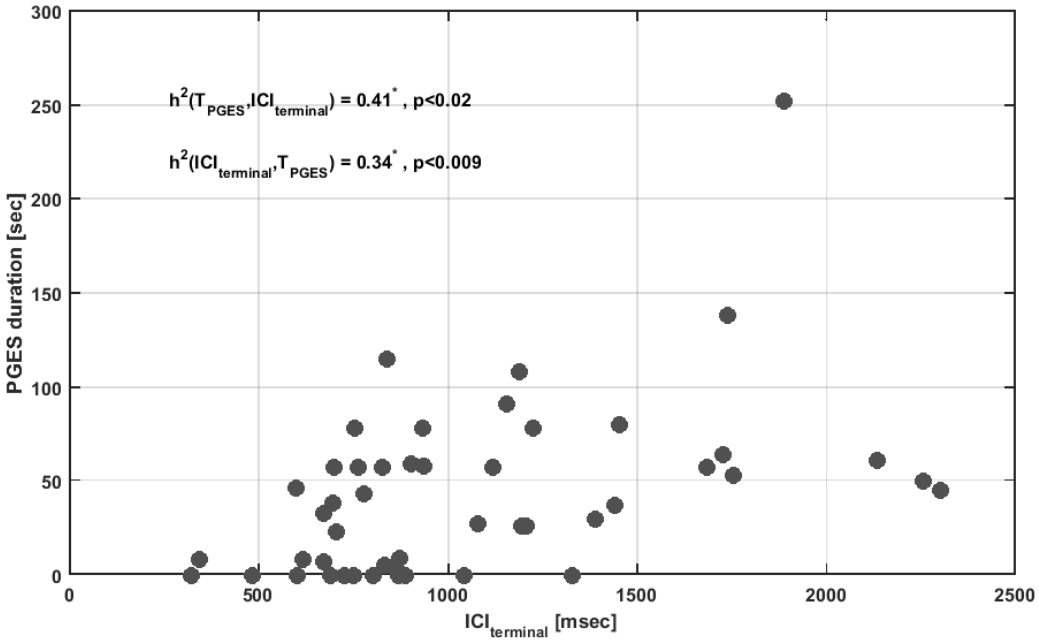

Figure 4.10: Relation between interclonic interval and PGES duration in EEG data*. Scatter plot showing the relation between the $ICI_{terminal}$ values (in msec, horizontal axis) and PGES duration (in seconds, vertical axis). Convulsive seizures that were not followed by a PGES event were accounted as 0 s. The non-linear association index h^2 was determined and shows a relatively small, but statistically significant functional relation (p<0.05) between PGES duration and $ICI_{terminal}$ in both directions.*

4.4 Discussion

The combination of computational modelling and human EEG recordings of convulsive seizures has revealed three important findings. 1) The probability distributions of the durations of ictal and postictal periods are indicative of deterministic processes. 2) The exponential increase of interclonic interval, observed during human seizures, may reflect a decrease in neuronal network connectivity that in our model leads to seizure termination and PGES. 3) The projected terminal interclonic interval ($ICI_{terminal}$) is associated with the occurrence and duration of PGES. These results are in agreement with the hypothesis that a neuronal mechanism that underlies transitions from ictal to postictal, and from postictal to normal states may be activated in response to total synchronisation during a convulsive seizure.

Gradual slowing of epileptic bursts and clonic frequency towards the end of seizures is frequently observed, but not fully understood (Conradsen *et al.*, 2013; Panayiotopoulos *et al.*, 2010; Truccolo *et al.*, 2011). Our findings suggest that this phenomenon may be related to plastic changes in connectivity. Several studies report on dynamical changes during the ictal state. Animal models of focal epilepsy have shown that the excitatory-inhibitory balance changes during a seizure, in line with the dynamics in this study (Boido *et al.*, 2014; Žiburkus *et al.*, 2013). Towards the end of a seizure, both excitatory and inhibitory neuron populations become increasingly active. This may lead to increased burst activity and longer inter-burst intervals (Boido *et al.*, 2014). Interneurons also receive strong excitatory input, leading to continuous activation of the inhibitory inputs to pyramidal cells, and seizure termination (Žiburkus *et al.*, 2013). Recent studies have shown changes in high-frequency oscillatory dynamics, and increased spatial and temporal correlation in human EEGs during the ictal state, providing additional evidence for plastic changes towards the end of a seizure leading up to seizure termination (Kramer *et al.*, 2012; Stamoulis *et al.*, 2013).

Transitions from ictal to postictal states are clinically important in view of SUDEP following PGES and status epilepticus. The successful modelling of the transition from ictal to postictal state in our neural mass model suggests that a PGES state can be caused by neuronal mechanisms alone, although other factors may contribute. Neuronal exhaustion was previously suggested as a possible mechanism of PGES, but seizure duration as such, was not associated with PGES in my sample and others,

making neuronal exhaustion an unlikely cause of seizure termination and PGES (Freitas *et al.*, 2013; Lamberts, Gaitatzis, *et al.*, 2013; Lamberts, Laranjo, *et al.*, 2013; Lhatoo *et al.*, 2010; Semmelroch *et al.*, 2012; Seyal *et al.*, 2012; Surges *et al.*, 2011). It is possible that several pathways lead to PGES. In addition to EEG suppression induced by diffuse cortical inhibition, EEG suppression can also be induced by hypoxia, hypotension, and asystole, which may all occur in the postictal state (Bozorgi *et al.*, 2013; van Dijk *et al.*, 2014; Massey *et al.*, 2014; Moseley *et al.*, 2013; Ryvlin *et al.*, 2013; Surges and Sander, 2012).

Our results show characteristics of global seizure dynamics, but cannot exactly predict which neurophysiological substrate causes both seizure termination and PGES. A variety of processes may be involved. An *in vitro* study showed that synchronous high-frequency firing of neurons, analogous to the ictal state, causes release of adenosine (Lovatt *et al.*, 2012). *In vivo,* adenosine concentration rises sharply during the last phase of a seizure in swine and humans, reaching a maximal level after seizure termination (Van Gompel *et al.*, 2014). A model in which the transition from high frequency to low frequency discharges in the course of a seizure is mediated by an increase in the Ca-dependent K^+ current, leading to an increase of extracellular K^+ has been proposed (Somjen *et al.*, 2008). The authors show that an overload of $[K^+]_o$, can initiate spreading depression, and thus termination of seizure discharges (Somjen *et al.*, 2008). Another computational study linked seizure termination and postictal depression to the complex interaction between sodium, potassium, and chloride concentrations (Krishnan and Bazhenov, 2011). These two studies demonstrate processes at a microscopic level, which are analogous to the transition from seizure to PGES at a macroscopic level, which we describe. These processes may account for the reported decrease in connectivity and excitability. The translation from the microscopic level of modelling to the macroscopic level is a matter for further study. Further investigations are needed to determine the exact role of these processes in causing seizure termination and PGES *in vivo*. We hypothesise that a neuronal seizure termination mechanism serves to restore normal function and to protect the brain from damage arising from neuronal exhaustion and metabolic depletion. Such a mechanism may also prevent status epilepticus or seizure clusters. If this "neuronal emergency brake" is activated too strongly or persistently, PGES occurs.

Excessive clonic slowing in relation to PGES may be considered a feature of a critical transition, in line with observations of slowing as a generic feature and possible early warning signal in systems approaching a critical transition or 'tipping point' (Scheffer *et al.*, 2009). The finding that PGES was always preceded by a marked decrease in clonic frequency is important as it may lead to the development of an algorithm for real-time interclonic interval detection of potentially fatal seizures using motion-detection sensors, including remote video detection (Kalitzin *et al.*, 2012, 2016).

Any computational model of complex systems such as the human brain can only account for a limited number of properties. Our model is an abstract representation of neuronal dynamics. It is, however, capable to predict relevant phenomena, such as the gradual change of the ictal state towards its termination. When using computational models it is essential to distinguish between *embedded* (created and pre-tuned) and *emergent* (predictive) properties of the model. In our model, the oscillatory state of the individual units is embedded, while the collective dynamics and the transitions between states are emergent properties, with potential predictive value. We consider the existence of oscillatory states, interpreted as model seizures and their deterministic termination mechanism as a built-in property. The existence of PGES states and their transient dynamics, however, are emergent properties of the collective system dynamics. The same holds true for the association between the duration of the PGES state and the value of the connectivity parameter g at seizure termination. These emergent properties can be qualitatively explained by the phase-space structure shown in figure 4.8, which can be interpreted as an emergent property in its entirety as it cannot be reduced to the dynamics of the individual units. The predictive power of our model is also due to its autonomous nature. Many computational models of the epileptic condition require the adjustment of their parameters in order to change behaviour from "seizure" to "normal". Such models can describe the individual states but will not provide predictions, or emergent features, from the dynamics of the transition between those states. Our model describes the transitions from ictal to postictal and back to a normal state as an autonomous process without any pre-defined parameter alterations. The only precipitating factor affecting the transitions is the stochastic noise present in the system.

Other types of plastic change may exist in addition to the plasticity of the connectivity

parameter we used in the model. We tested several types of plasticity mechanisms, affecting either the unit excitability, or the inter-unit connectivity or both. All lead to (1) deterministic seizure termination, and (2) a transient postictal state with suppressed activity and excitability. In all cases, the duration of the postictal supressed state was associated with the terminal value of the plasticity parameter. It is, however, the particular choice of equation (2), and the interaction term in equation (1), that relates the interclonic interval increase during the seizure to the change in connectivity parameter g. Our model may be used as a starting point to reconstruct the exact properties of the mechanism of seizure termination, using a more detailed model. Our results may not apply to all seizure types. A different type of model, for example, predicts a logarithmic (ICI~log(t)) evolution of the interclonic intervals preceding a homoclinic bifurcation at seizure offset (Jirsa et al., 2014). It was validated in a clinical sample, which appears to have been selected based on different seizure criteria as ours. The underlying pathophysiological mechanisms of seizure termination may therefore differ. This may explain the exponential instead of a logarithmic evolution of the interclonic interval in our sample. Previous work showed that the probability distribution of the duration of absence seizures in humans and rodent and computational models of epilepsy can, in certain cases, also follow a stochastic pattern (Suffczynski *et al.*, 2006). This suggests that the termination mechanism is defective in certain circumstances, causing seizures to terminate due to random fluctuations.

The sample size of our human EEG data is limited and surface EEG for postictal assessment presents some drawbacks. Artefacts (e.g. nursing interventions, EMG and breathing activity) may have contaminated the EEG, thereby preventing precise estimation of PGES duration. Despite being a well-defined neurophysiological state that is easier to quantify than postictal slowing in general, PGES duration is inevitably a semi-exact outcome measure. One way to circumvent this is to use intracranial EEG recordings, but because of sparse spatial sampling this will lack a global measure of cortical activity. In my sample, AEDs were tapered in the course of seizure monitoring. Such tapering may increase the occurrence of PGES (Lamberts, Gaitatzis, *et al.*, 2013), and may theoretically alter mechanisms of seizure termination. Despite these limitations, this study demonstrates the power of combining computer modelling and neurophysiological observations in formulating testable hypotheses leading to new approaches to elucidate epileptic seizure mechanisms in human EEG data.

5

The topographical distribution of epileptic spikes in juvenile myoclonic epilepsy with and without photosensitivity

"To observe without thinking is as dangerous as thinking without observing."[5]

5.1 Juvenile myoclonic epilepsy

Juvenile myoclonic epilepsy (JME) is a type of genetic epilepsy characterised by myoclonic jerks shortly and generalised tonic clonic seizures. The diagnosis is based on the clinical presentation, that includes myoclonic jerks shortly after awakening, normal intelligence and an age of onset between 10 and 25 years. The diagnosis is confirmed when at the time of the myoclonic jerks, the electroencephalographic (EEG) recording shows a normal background pattern and ictal bilaterally symmetric, high amplitude polyspikes and waves with frontocentral dominance (Janz, 1985; Kasteleijn-Nolst Trenité *et al.*, 2013). The interictal EEG shows generalised 3-6Hz spike-wave or polyspike-wave activity, also with frontocentral dominance (Janz, 1985; Kasteleijn-Nolst Trenité *et al.*, 2013; Koepp *et al.*, 2014). Focal abnormalities such as single spikes, spike-and-wave complexes, and slow waves are seen in 30-45% of cases (Aliberti *et al.*, 1994; Lancman *et al.*, 1994; Seneviratne *et al.*, 2014), contributing to diagnostic delay (Panayiotopoulos *et al.*, 1991). Over a third of people with JME also have absence

[5] Ramón y Cajal S., (translation by Swanson N, Swanson LW). *Advice for a young investigator*. The MIT Press, Cambridge, MA, 1999, p118

seizures (Beghi *et al.*, 2006). At least thirty percent of people with JME have a photoparoxysmal response (PPR). In some, flashing lights can trigger myoclonic jerks or generalised tonic clonic seizures (Appleton *et al.*, 2000; Wolf and Goosses, 1986). The PPR is an abnormal response to intermittent photic stimulation. There are four types of PPR, classified by Waltz *et al* (1992): (I) spikes within the occipital rhythm, limited to the occipital regions (II) parieto-occipital spikes with a biphasic slow wave, (III) parieto-occipital spikes with a biphasic slow wave and spread to the frontal region, and (IV) generalised spikes and wave or polyspikes and wave (Waltz *et al.*, 1992). The Waltz types (I) and (II) responses are generally seen as unrelated to epilepsy (Kasteleijn-Nolst Trenité *et al.*, 2001; Waltz *et al.*, 1992). The prevalence of PPR in the general population is estimated around 1.5% (Koeleman *et al.*, 2013). Type (III) and (IV) are considered abnormal. Especially type (IV) appears to be correlated with epilepsy (Kasteleijn-Nolst Trenité *et al.*, 2001). Several recent brain imaging studies have shown different connectivity patterns in people with JME leading to the hypothesis that JME is a network epilepsy (Bartolini *et al.*, 2014; Koepp, 2005; Koepp *et al.*, 2014; Vollmar *et al.*, 2012). In people with JME+PPR, the connectivity between the supplementary motor area and occipital cortex was stronger than in people with PPR (Vollmar *et al.*, 2012). Although this difference did not reach statistical significance, this could provide a tentative explanation for the fact that photic stimulation can elicit myoclonic jerks in people with JME+PPR (Vollmar *et al.*, 2012). I hypothesise that interictal epileptic discharges may be linked to altered connectivity, and that people with JME+PPR may have more focal interictal epileptic abnormalities in the posterior regions than people with JME-PPR.

My colleagues and I tested this hypothesis in the following study by comparing the locations of the maximum of interictal generalised activity and localised epileptiform abnormalities between people with JME+PPR type (III) or (IV) and people with JME-PPR (including PPR type (I) and (II)).

5.2 Methods

5.2.1 EEG selection

I obtained EEG recordings of people with JME by screening the electronic EEG report databases of the departments of Clinical Neurophysiology of SEIN and the University Medical Center Utrecht (UMCU), using the keywords “JME” and the Dutch word for

juvenile ("juveniele"). The search encompassed EEG recordings done between 1999 and early 2015 for SEIN, and 2010 to 2015 for the UMCU. The study was approved by the medical ethical committee of the UMCU, which judged informed consent unnecessary as it pertained a retrospective analysis of data collected for clinical purposes. Data were coded for analysis.

Only EEG recordings of people who were not taking anti-epileptic drugs (AED) were included. Inclusion criteria were: (a) a confirmed diagnosis of JME or a confirmed diagnosis of genetic epilepsy with a strong suspicion of JME, based on the EEG or clinical presentation; (b) at least one drug naïve EEG recording available; and (c) photosensitivity tested using intermittent photic stimulation, either during the EEG recording that was evaluated for the current study, or in a previous EEG recording. Exclusion criteria were: (a) incomplete records; (b) any history of neurological comorbidity that could influence the diagnosis of JME; and (c) any abnormalities on Magnetic Resonance Imaging. Duplicates and reports other than EEG reports were excluded. Clinical information was retrieved from the hospital files.

5.2.2 EEG recordings

At SEIN, the 32-channel EEG recordings were recorded at 500Hz using Stellate Harmonie (Stellate inc, Montreal, Canada) and a Grass photic stimulator (PS33+, Grass Products, Quincy, Mass., USA) until 2012 and subsequently at a 512Hz sample frequency with a SystemPlus Micromed EEG system (Micromed SD 16 DC, Treviso, Italy) and photic stimulator (Micromed, Flash 10S Treviso, Italy). At the UMC Utrecht EEGs were recorded using the Micromed Smart Acquisition Module amplifier (Micromed, Treviso, Italy), at a sample frequency of 512Hz and intermittent photic stimulation was performed using the Micromed stimulator. In both centres, electrodes were placed according to the international 10-20 system, with additional electrodes on the ear lobes (A1 and A2). Conventional 10mm Ag-AgCl electrodes were used. EEG recording was performed according to the standard clinical protocol, with or without sleep deprivation.

5.2.3 EEG analysis

After selecting the EEG reports, we retrieved the original EEG recordings. They were re-evaluated by an experienced neurophysiology technician (Willy Spetgens) who was familiar with the reporting style of both centres, and who was unaware of the research

question. The localised (focal) epileptiform abnormalities and their location outside intermittent photic stimulation or hyperventilation were assessed. Localised abnormalities were defined as paroxysmal focal activity, localised (poly)spike-and-slow-wave activity and (poly)sharp-and-slow-wave complexes (Noachtar *et al.,* 1999*)*. We divided the EEGs into four groups based on the location of the interictal localised abnormalities: No localised abnormalities at all (L-); localised abnormalities present, but not involving the posterior regions (L_{POST-}), see figure 5.1 A; localised abnormalities present, also in posterior regions (L_{POST+}), see figure 5.1 B; localised abnormalities present only in the posterior regions ($L_{|POST|}$), see figure 5.1 C. The EEGs were also divided in terms of the maximum amplitude of generalised or bilateral synchronous discharges outside intermittent photic stimulation as follows: No generalised abnormalities at all (G-); generalised discharges with maximal amplitudes in the anterior regions ($G_{ANT>POST}$), see figure 5.1 D; generalised discharges with maximal amplitudes in the posterior regions ($G_{POST>ANT}$); bilateral synchronous discharges without a clear or alternating maximum ($G_{ANT=POST}$), see figure 5.2; bilateral synchronous spike-wave discharges limited to the anterior regions ($G_{|ANT|}$); bilateral synchronous spike-wave discharges limited to the posterior regions ($G_{|POST|}$). The EEG reports were also divided according to the presence of PPR, defined as an abnormal posterior response spreading to anterior regions (Waltz criteria III or IV)(Waltz *et al.*, 1992). Waltz I and II were included in the JME-PPR group. People were divided into JME-PPR and JME+PPR based on all available EEG reports, so the distinction between JME-PPR and JME+PPR was not only based on the studied EEG recording. We compared the number and type of localised discharges (groups L), and the maximum of the generalised SW discharges (groups G), between the JME+PPR and JME-PPR groups.

5.2.4 Statistical analysis

I compared the clinical characteristics between the people seen at the two centres, and between the JME+PPR and JME-PPR groups using Chi^2 test and Fishers exact test. I compared the number of people with JME+PPR and JME-PPR with discharges not involving the posterior regions (L_{POST-} and $G_{|ANT|}$), to the number of people with JME+PPR and JME-PPR with discharges involving the posterior regions (L_{POST+}, $L_{|POST|}$, and $G_{ANT=POST}$, $G_{ANT>POST}$, $G_{POST>ANT}$, $G|_{POST|}$,) using a Chi^2 test and Fishers exact test. I considered a p-value below 0.05 to indicate significance.

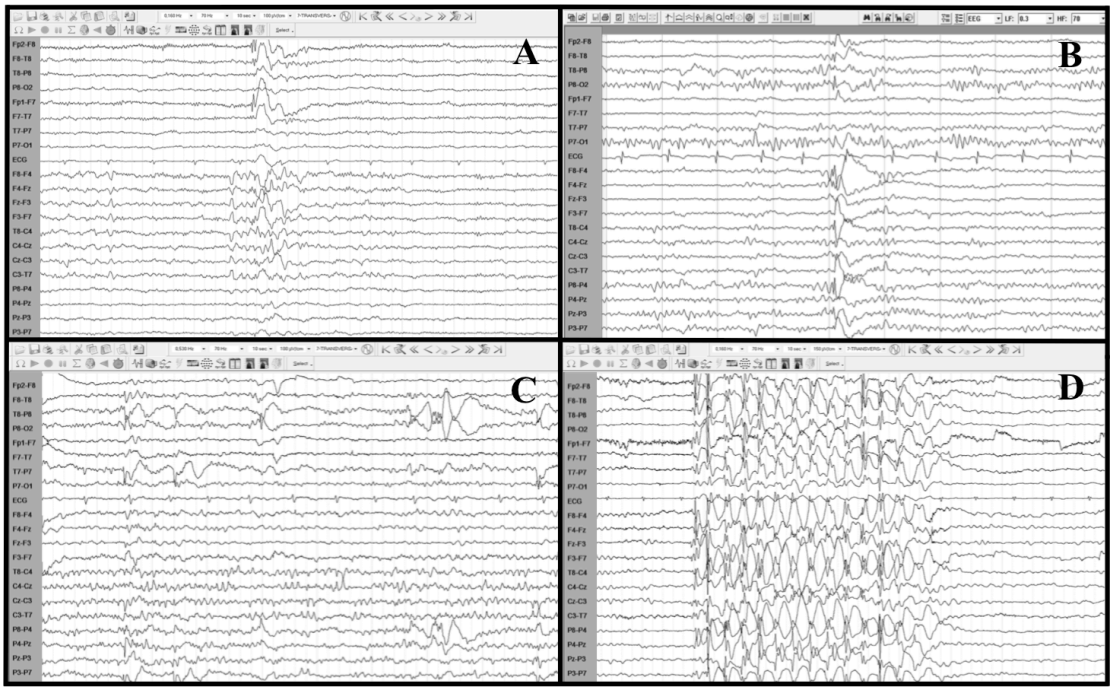

Figure 5.1: Examples of localised and generalised EEG discharges outside intermittent photic stimulation. ***A:*** *L_{POST-}, localised (poly)sharp-and-slow-wave complex not involving the posterior regions (drowsy). Filter settings: low pass: 0.160Hz, high pass: 70Hz, scale: 100 µV/cm.* ***B:*** *L_{POST+} localised spike-and-slow-wave activity involving posterior regions (eyes closed). Filter settings: low pass: 0.300Hz, high pass: 70Hz, scale: 100 µV/cm.* ***C:*** *$L_{|POST|}$ spike-and-slow-wave complex limited to the posterior regions (eyes open). Filter settings: low pass: 0.530 Hz, high pass: 70Hz, scale: 100 µV/cm.* ***D:*** *$G_{ANT>POST}$, bilateral synchronous (poly)sharp-and-slow-wave and spike-and-slow-wave discharges with anterior maximum. Filter settings: low pass: 0.160Hz, high pass: 70Hz, scale: 150 µV/cm.*

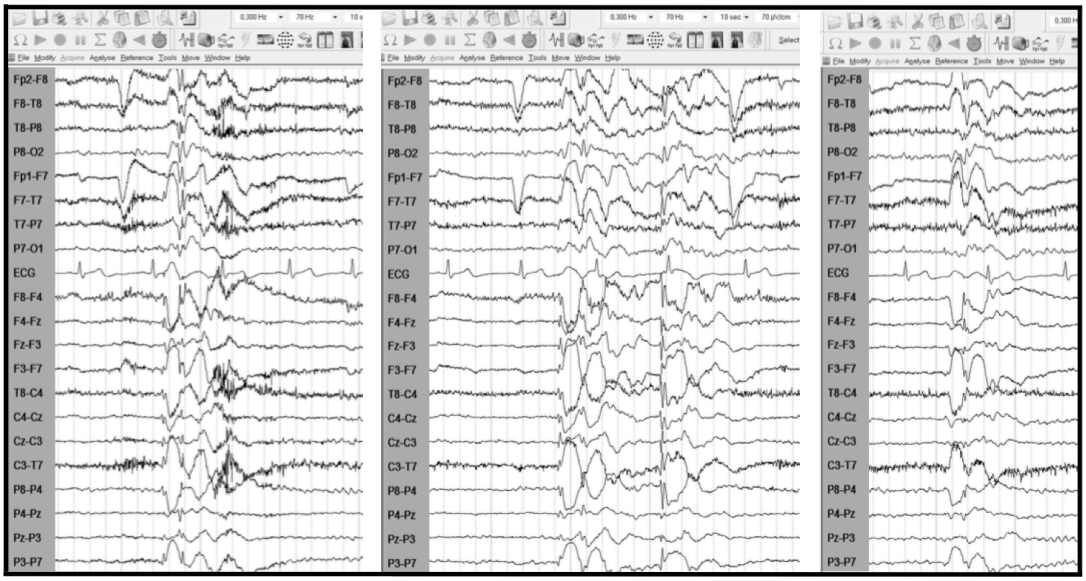

Figure 5.2: Example of generalised EEG discharges outside intermittent photic stimulation without a clear maximum. *Three events from the same patient, showing bilateral synchronous sharp-and-slow-wave and spike-and-slow-wave discharges with an alternating maximum ($G_{ANT=POST}$). Filter settings: low pass: 0.300Hz, high pass: 70Hz, scale: 70 µV/cm.*

5.3 Results

I retrieved 180 EEG reports mentioning JME from between 1999 to April 2015 in SEIN. In the UMCU, we found 60 reports from EEG recordings done between 2010 and 2015. Of these, 159 from SEIN and 36 from the UMCU did not meet the inclusion criteria, for example because the EEG was done under medication or not suggestive of JME. Three original recordings from the UMCU and three from SEIN were unavailable. A total of 39 (21 from UMCU, 18 from SEIN) recordings were included in this study for re-evaluation and statistical analysis.

5.3.1 Subject characteristics

Age, gender, occurrence of generalised tonic clonic seizures, absence seizures, PPR response, diagnosis, and diagnosis using Magnetic Resonance Imaging did not differ between the centres (table 5.1). The age of onset of epilepsy was not available for one person. Myoclonic jerks were described more often in reports from SEIN than those from the UMCU, and neurologists from SEIN more often reported a definite diagnosis of JME than their colleagues at the UMCU. The prevalence of PPR did not differ between the two centres.

Table 5.1: Group characteristics of people with JME per centre.

	UMCU (n=22)	**SEIN** (n=17)	**test, p-value**
Female (N)	12 (55%)	10 (59%)	χ^2, 0.79
Mean age at EEG (years)	17.95 (SD=9.11)	20.35 (SD=5.56)	MW, 0.07
Mean age of onset (years)	14.71 (SD=4.16)	16.75 (SD=3.11)	MW, 0.26
Myoclonic jerks (N)	14 (64%)	17 (100%)	F, <0.01*
Generalised seizures (N)	19 (86%)	11 (65%)	F, 0.14
Absences (N)	4 (18%)	4 (24%)	F, 0.71
Confirmed diagnosis JME (N)	8 (36%)	12 (71%)	χ^2, 0.03*
Diagnosis probably JME (N)	14 (63%)	5 (29%)	χ^2, 0.03*
PPR (N)	9 (41%)	9 (53%)	χ^2, 0.46
Photosensitivity in daily life (N)	3 (14%)	1(6%)	F, 0.62
No MRI done (N)	12 (55%)	11 (65%)	F, 0.74
Negative MRI known (N)	10 (45%)	6 (35%)	F, 0.74
Epilepsy in first degree family (N)	0 (0%)	1 (6%)	F, 0.44
Epilepsy in second degree family (N)	18 (81%)	13(76%)	F, 0.71

*PPR= photoparoxysmal response, MRI= Magnetic Resonance Imaging. Percentages are shown in brackets *statistical significance at a level of p<0.05. χ^2 = Chi² test, MW = Mann-Whitney U test, F= Fisher's exact test.*

The clinical characteristics of the JME+PPR and JME-PPR groups are listed in table 5.2. Gender, age of onset of epilepsy, age at EEG recording, occurrence of myoclonic jerks, generalised tonic clonic seizures, history of epilepsy in the family, and current diagnosis did not differ between JME+PPR and JME-PPR groups.

5.3.2 EEG analysis

The EEG characteristics for JME+PPR and JME-PPR are shown in table 5.3. The background pattern was normal in all recordings. In several cases, intermittent photic stimulation was not performed during the re-evaluated EEG recording because the presence or absence of PPR had been confirmed previously. In one person, intermittent photic stimulation was not completed because of a strong epileptiform reaction and risk of provoking a generalised tonic clonic seizure. Localised abnormalities (L+) were present in 35 of the 39 EEG recordings (table 5.3). The prevalence of localised abnormalities did not significantly differ between people with JME+PPR and people with JME-PPR. In people with JME-PPR, localised abnormalities without posterior involvement (L_{POST-}) were seen more often than in people with JME+PPR, while localised abnormalities involving the posterior areas were seen more often in people with JME+PPR ($p<0.01$). In four people with JME+PPR but none of the people with JME-PPR, localised abnormalities were limited to the posterior regions ($L_{|POST|}$).

Table 5.2: Group characteristics of people with JME-PPR+ and JME-PPR.

	PPR+ (n=18)	**PPR-** (n=21)	**test, p-value**
Female (N)	13 (72%)	9 (43%)	χ^2, 0.07
Mean age at EEG (years)	18.1 (SD=6.5)	19.8 (SD=7.4)	MW, 0.51
Mean age of onset (years)	15.9 (SD=3.4)	15.6 (SD=4.3)	MW, 0.78
Myoclonic jerks (N)	14 (78%)	17 (80%)	F, 1.00
Generalised seizures (N)	14 (78%)	16 (76%)	F, 1.00
Absences (N)	3 (17%)	5 (24%)	F, 0.70
Confirmed diagnosis JME (N)	10 (56%)	10 (48%)	χ^2, 0.62
Diagnosis *probably* JME (N)	8 (44%)	11 (52%)	χ^2, 0.62
Photosensitivity in daily life (N)	3 (17%)	1 (5%)	F, 0.31
Negative MRI known (N)	6 (33%)	10 (48%)	F, 0.51
No MRI done (N)	12 (67%)	11 (52%)	F, 0.51
Epilepsy in 1st degree family (N)	1 (6%)	0 (0%)	F, 0.46
Epilepsy in 2nd degree family (N)	2 (11%)	6 (29%)	F, 0.25

PPR= photoparoxysmal response, MRI= Magnetic Resonance Imaging. χ^2 = Chi² test, MWU = Mann-Whitney U test, F= Fisher's exact test.

Generalised and bilateral synchronous spike-wave discharges were present in 14 of the 18 people with JME+PPR and 17 of the 21 people with JME-PPR. There was no difference in involvement of the occipital lobe in both groups. Most people had generalised spike-wave discharges with an anterior maximum ($G_{ANT>POST}$). In five people with JME+PPR, the onset of the generalised spike-wave discharges could be delineated. In all five, it had a clear posterior onset during intermittent photic stimulation. In two of those, there was also a posterior spike-wave discharge onset outside intermittent photic stimulation, while in the other three the onset could not be discerned.

Table 5.3: EEG comparison between JME-PPR+ and JME-PPR.

	PPR+ (n=18)	**PPR-** (n=21)	**test, p-value**
Localised abnormalities outside posterior areas (L_{POST-})	**4 (22%)**	**17 (76%)**	**χ^2, 0.002***
Localised involving posterior areas (L_{POST+}, $L_{\|POST\|}$)	**10 (55%)**	**4 (19%)**	
No localised abnormalities (L-)	4 (22%)	0 (0%)	
Any localised abnormalities (L_{post-} , L_{post+}, $L_{\|post\|}$)	14 (78%)	21 (100%)	
Localised abnormalities also in posterior areas ($L_{post+,}$)	6 (33%)	4 (19%)	
Localised abnormalities only in posterior areas ($L_{\|post\|}$)	4 (22%)	0 (0%)	
Generalised SWD's limited to anterior areas ($G_{\|ANT\|}$)	**0 (0%)**	**3 (14%)**	**F, 0.232**
Generalised SWD's involving posterior areas	**14 (78%)**	**14 (67%)**	
No generalised discharges (G-)	4 (22%)	4 (19%)	
Bilateral synchronous generalised SWD's ($G_{ANT=POST}$)	3 (17%)	1 (5%)	
Generalised SWD's anterior maximum ($G_{ANT>POST}$)	9 (50%)	13 (62%)	
Generalised SWD's posterior maximum ($G_{POST>ANT}$)	2 (11%)	0 (0%)	
Generalised SWD's limited to posterior areas ($G_{\|POST\|}$)	0 (0%)	0 (0%)	

*PPR= photoparoxysmal response. SWDs=spike-wave discharges. Percentages are shown in brackets. *statistically significant at a level of p<0.05 with χ^2 test. F= Fisher's exact test.*

5.4 Discussion

This study shows that people with JME+PPR and people with JME-PPR have a different distribution of localised interictal EEG abnormalities. The total number of localised abnormalities does not differ significantly between people with JME+PPR and people with JME-PPR, but people with JME-PPR in my sample had significantly less localised abnormalities involving the posterior regions than people with JME+PPR. There was no difference in the distribution of generalised spike-wave discharges between people with JME+PPR and JME-PPR. Our findings corroborate with several recent findings of

altered excitability and connectivity between different brain regions in JME (Brigo, Storti, Benedetti, *et al.*, 2012; Vollmar *et al.*, 2012).

The exact mechanism underlying PPR is unknown. It was proposed that it is caused by the hyperexcitability of the primary visual cortex (Brigo *et al.*, 2013; Siniatchkin *et al.*, 2007). Increased connectivity between the occipital areas and the supplementary motor area may enable discharges to spread rapidly to other regions of the brain, as shown by an imaging study in people with JME compared to healthy controls (Vollmar *et al.*, 2012). This notion is supported by the fact that intermittent photic stimulation in people with photosensitive epilepsy resulted in temporary increased excitability and reduced inhibition of the motor cortex (Groppa *et al.*, 2008; Strigaro *et al.*, 2013; Strigaro, Falletta, *et al.*, 2015). The difference in localisation of focal abnormalities in the EEGs of people with JME+PPR and JME-PPR that we report may be an expression of the hyperexcitability of the occipital cortex. A magnetoencephalography study in people with idiopathic photosensitive epilepsy (old classification) and healthy controls showed that there is increased phase clustering in the gamma frequency band at rest and before the onset of PPR in people with photosensitive epilepsy (Parra *et al.*, 2003). It was hypothesised that in people with epilepsy, but not in controls, intermittent photic stimulation entrains neuronal networks, leading to excessive synchrony, which may be apparent on the EEG as spike-wave discharges. Increased connectivity between the posterior and anterior regions may cause these localised discharges to develop into generalised spike-wave discharges and potentially to seizures. The finding that there is a similar distribution of the maximum of generalised spike-wave discharges in both groups may be explained by the fact that epileptic discharges likely spread along the superior longitudinalis fasciculus, which projects into the frontal lobe.

Photosensitivity is also seen in people without epilepsy and is thought to be a heritable trait, but so far no specific genes have been identified. CHD2 mutations have been linked to photosensitive epilepsy (Galizia *et al.*, 2015). BRD2 mutations have been linked to both PPR and JME in one study (Pal *et al.*, 2003) but not in others (Cavalleri *et al.*, 2007; de Kovel *et al.*, 2007; Lorenz *et al.*, 2006). It is possible that both PPR and JME are caused by polygenetic mechanisms and that different combinations of genetic variations can lead to slight variations of the clinical phenotype (Taylor *et al.*, 2004).

This study is limited by the relatively small sample size and the fact that clinical EEG recordings offer a limited time window. Most EEG recordings in this study lasted 24 hours at most. It is therefore possible that characteristics, which only appear at a certain time of the day, have been missed. For example, PPR and focal abnormalities and generalised spike-wave discharges in JME may be more prevalent in morning EEG recordings (Kasteleijn-Nolst Trenité *et al.*, 2007; Labate *et al.*, 2007). Between 1999 and 2015 two different stroboscopes were used for intermittent photic stimulation in SEIN (Grass and Micromed). Some people were tested for photosensitivity with both and had a PPR only with one of the stroboscopes (usually Grass). Some people may thus erroneously have been classified as JME-PPR since the introduction of the Micromed stroboscope. As this concerns five people, we do not expect that this has a significant impact on the results presented, but should be kept in mind whenever testing for photic sensitivity (Specchio *et al.*, 2011). There was a female preponderance (72%) in my sample in the JME+PPR group, which did not reach statistical significance. Previous studies showed that women with JME were more often photosensitive than men with JME (Wolf and Goosses, 1986). Localised abnormalities were seen in 89% of the EEGs in my sample. This is higher than reported in previous studies, and may have been caused by referral bias. Localised epileptiform abnormalities can complicate the diagnosis of JME, leading to more referrals to specialised centres such as ours (Aliberti *et al.*, 1994; Lancman *et al.*, 1994). Epileptiform EEG events are dynamic and can vary considerably within the same person. Perhaps the most important factor is that JME is a clinical diagnosis with a polygenetic aetiology. This study is based on clinical and EEG assessments and it is likely that my sample is heterogeneous. Several studies have described different clinical presentations of the JME spectrum (Martínez-Juárez *et al.*, 2006; Taylor *et al.*, 2004). Interestingly, there may be a considerable overlap between idiopathic photosensitive occipital lobe epilepsy and JME in certain cases (Taylor *et al.*, 2004). Visual aura and conscious head version are classically associated with genetic photosensitive occipital lobe epilepsy, but are also reported in JME. It is possible that people with this phenotype have more focal EEG abnormalities in the posterior brain regions.

For successful treatment, it is paramount to differentiate JME from focal epilepsy. Focal EEG abnormalities in JME, in some cases combined with symptoms such as visual auras and conscious head version, may lead to an erroneous diagnosis of focal

epilepsy for which carbamazepine would be the treatment of choice (Aliberti *et al.*, 1994; Lancman *et al.*, 1994). Carbamazepine, however, aggravates myoclonic jerks and potentially increases the number of generalised tonic clonic seizures (Thomas *et al.*, 2006).

This study underlines that localised EEG abnormalities are a common feature in JME, and shows that people with JME-PPR have less localised EEG abnormalities involving the posterior areas than people with JME+PPR. Defective inhibition and increased excitability of the occipital cortex may explain this phenomenon. Whether more advanced ways of EEG analysis, or other forms of stimulation can reveal these epileptic networks in JME will be addressed in **Chapter 6** and **8.**

6

Measuring synchronisability and multistability with TMS in healthy controls, Juvenile Myoclonic Epilepsy and Migraine

Hypotheses, participants and methods

"The far-reaching importance of attention to detail in technical methodology is perhaps demonstrated more clearly in biology than in any other sphere."[6]

6.1 Hypotheses and aims

In the previous chapters I have discussed several features of epilepsy: multistability, increased potential for synchronisation (synchronisability), and higher cortical excitability. Previous studies have shown that anti-epileptic drugs (AEDs) can lower the cortical excitability in people with epilepsy and that this may correlate with seizure freedom. Cortical excitability can be measured with Transcranial Magnetic Stimulation (TMS), which is described in **chapter 2**. In the next three chapters, I describe a study in which my colleagues and I measured different variables associated with cortical excitability in healthy controls, in people with juvenile myoclonic epilepsy (JME), and in people with migraine with aura. Firstly, we aimed to develop an automated method to calculate the motor threshold based on TMS and electromyography (EMG). I compare motor thresholds obtained with three different methods between healthy

[6] Ramón y Cajal S., (translation by Swanson N, Swanson LW). *Advice for a young investigator*. The MIT Press, Cambridge, MA, 1999, p16

controls, people with JME and people with migraine. Second, I did a paired-pulse paradigm in the healthy controls and the JME group to assess the long-interval intracortical inhibition (LICI) recovery curve. I compare my findings to findings described in the literature (**chapter 2).** Lastly, I show that TMS-electroencephalography (EEG) variables may be used to measure multistability and synchronisability in people with epilepsy. I explore how these new TMS-EEG variables could be used to predict the response to AEDs. In the previous chapters, I showed that migraine shares some features with epilepsy (**chapter 2.1**), such as the paroxysmal nature of the condition, and the change of the brain state(s) during an attack (**chapter 4**). Epilepsy and migraine often co-occur in the same person (**chapter 3**), and triggers for attacks can be the same in migraine and epilepsy (**chapter 4**). The comparison of TMS-EEG variables of synchronisability and multistability in people with migraine with aura, JME, and controls, may help to further elucidate the common pathophysiological mechanisms underlying these two conditions.

6.1.1 Resting motor threshold calculation

The studies discussed in **Chapter 2.2** show how essential it is to reliably and precisely estimate the resting motor threshold (rMT) when using TMS, as many other parameters depend on it. In this study, I investigate an automated method to calculate the rMT. Classically, the rMT is defined as the "lowest stimulus intensity (given as percentage of maximal stimulator output) that is required to induce a motor evoked potential (MEP) of at least 50 uV in 5 out of 10 trials" (Groppa *et al.*, 2012). This method is commonly used, but there are several issues. First, the MEP is highly variable due to fluctuations in the excitability of the brain (Giambattistelli *et al.*, 2014). Second, the rMT has a probabilistic nature, and can therefore not be estimated reliably with this method (Tranulis *et al.*, 2006). Third, there is no clear algorithm to estimate the rMT (Tranulis *et al.*, 2006). Several alternative methods have been proposed: the two-threshold method estimates the arithmetic mean of a lower (highest stimulus intensity that does not elicit a MEP) and higher threshold (lowest stimulus intensity that elicits a MEP in 100% of trials) (Mills and Nithi, 1997). The adaptive method is based on parameter estimation through sequential testing and maximum likelihood regression (Awiszus, 2003). In this method, the relationship between TMS stimulus intensity, and probability of eliciting a MEP, is modelled on an S-shaped metric function. The model predicts the TMS intensity that has a 50% probability of evoking a MEP for every

stimulus. The supervised parametric method is based on the sigmoidal relationship between increasing stimulus intensity and MEP probability. The rMT is estimated by fitting a sigmoid curve using the least mean squares method (Tranulis *et al.*, 2006). Alternatively, a stimulus response curve can be constructed using a Boltzmann sigmoid function (Mathias *et al.*, 2014). Although adaptive methods are more accurate than fixed-stimulus methods, they require more stimuli than fixed-stimulus methods and specialist software (Tranulis *et al.*, 2006). In addition, offline analysis can be challenging, as the unpredictable changes in stimulation intensity have to be taken into account. Ideally, a paradigm to determine the rMT should be exact, objective, quick, and reliable while offering the possibility of offline analysis of the data. In this work, I propose an extension of the parametric estimation that could potentially fulfil these requirements, based on the idea that two points in a sigmoidal function could correspond to a "threshold": the steepest point of the curve, or the "take-off" point, where the slope of the curve first starts to increase. The steepest point of a sigmoid curve can be found by calculating the maximum of the first derivative of the curve. The "take-off" point is the point where the second derivative of the curve is maximal. I compare the rMT estimated in the traditional way, using visual estimation, with the thresholds based on the maximum values of the first and second derivatives of the sigmoid fit of the stimulus response curves between controls, people with JME (with and without medication), and people with migraine.

6.1.2 Long-interval Intracortical Inhibition (LICI)

LICI curves are a promising biomarker for epilepsy (**chapter 2.2**). Whether they can be used as such on an individual level critically depends on the inter- and intra-individual variability of these curves. In **chapter 7,** I will show our LICI results in healthy controls and people with JME. I will address the issue of inter- and intra-individual variability and compare my results to existing literature.

6.1.3 TMS-EEG measures of synchronisability and multistability

In **chapter 2.2**, I also introduced TMS-EEG, a novel technique that has great potential to assess cortical excitability in epilepsy and other (neurological) conditions. So far, TMS-EEG studies have focused on the analysis of the TMS-evoked potential (TEP), or on eliciting epileptiform discharges with TMS to identify aberrant excitability or connectivity in epilepsy, but it has proven challenging to quantify changes between people with epilepsy and healthy controls (Del Felice *et al.*, 2011; Julkunen *et al.*, 2013;

Kimiskidis *et al.*, 2013; Shafi *et al.*, 2015; Valentin *et al.*, 2008). To my knowledge this is the first TMS-EEG study in migraine. I take a different approach from the TEP analysis and quantify evoked phase synchrony and non-linearity of the EEG response to single-pulse TMS. This method is inspired by a study that showed that people with photosensitive epilepsy display increased phase synchrony in the gamma band, compared to healthy controls in magnetoencephalography recordings in response to photic stimuli (Parra *et al.*, 2003). I now investigate whether TMS-evoked phase synchrony can be measured in the surface EEG, and whether this parameter differs between people with JME, people with migraine, and healthy controls. I also investigate the TMS-EEG response-curve, as it may provide information about multi-stability, or the propensity of the brain to be in paroxysmal pathological state. A linear TMS-EEG stimulus-response curve would be a sign of a stable system, while a curve that deviates from the linear fit is a sign of multistability **(chapter 4)**. The combination of increased synchronisability and multistability paramaters may contribute to a state of altered cortical excitability that characterises migraine and epilepsy.

6.2 Participants

6.2.1 Juvenile Myoclonic Epilepsy

Participants were recruited from the outpatient clinic of SEIN, Heemstede, The Netherlands, and from outpatient departments of hospitals in the Amsterdam region. The diagnosis of JME was based on the clinical history and interictal EEG recording. People aged 12 years and over, with a history of myoclonic seizures, at least one generalised tonic-clonic seizure, and who were either starting or tapering AEDs were included. People with co-morbid migraine were excluded.

6.2.2 People with migraine with visual aura.

People with migraine with visual aura were recruited in SEIN, and the Leids Universitair Medisch Centrum, Leiden. The diagnosis was based on the clinical history and the International Classification of Headache Disorders (ICHD-3) of the International Headache Society (Headache Classification Committee of the International Headache Society, 2013). Participants aged 18 years and over with migraine headaches, and a visual aura preceding the headaches in at least 30% of the attacks, were included in the study. Participants had to have at least one migraine

attack per year, at least one in the year preceding the study, and no more than eight attacks or 15 headache days per month. People with a history of epilepsy and people who used prophylactic medication were excluded, as were people with migraine without aura and aura sans migraine (no headache).

6.2.3 Controls

Healthy volunteers aged 12 years or over were recruited locally through digital and paper adverts. People with a history of epilepsy or migraine were excluded.

6.2.4 Exclusion criteria

Pregnant women and people with diabetes mellitus, people with a psychiatric condition, and people who used medication that could affect cortical excitability (such as psychoactive drugs and b-blockers) were excluded from the study. All participants gave written informed consent. Consent was also obtained from the parents or carers of participants younger than 18. The ethics committee of the Erasmus University Medical Centre, Rotterdam approved the study.

6.3 Stimulator and EMG and EEG recordings

6.3.1 Transcranial Magnetic Stimulation

Participants were screened for contra-indications to TMS, neurological conditions other than JME or migraine and other exclusion criteria. They were asked not to smoke, take drugs, or drink alcohol or coffee 12 hours preceding the measurement and to maintain a normal sleep pattern the night prior to the measurement. Magnetic Stimulation was performed with a MagPro X100 magnetic stimulator (Magventure, Denmark), a 14cm diameter parabolic circular coil (type MMC-140), and a placebo coil (type MCF-P-B65). Muscle activity was monitored using real-time visual feedback of the EMG. Measurements were conducted between 09.00AM and 04.00PM and spread evenly between AM and PM in the participant groups. Measurements were repeated after 10-12 months in controls to assess long-term reproducibility, and 6-8 weeks after medication changes in JME. People with migraine were only measured once. Repeated measurements were done at the same time of day.

As stated in the ethical protocol and informed consent, participants could leave the study at any time and for any reason. Investigations always took place with two investigators present, of which one was a physician who was monitoring the EEG

recording during the TMS session for signs of drowsiness. During sessions with participants with JME, a trained EEG technician monitored the EEG to alert the investigators to early signs of seizures, in which case stimulation was stopped immediately. The investigations took place during office hours, when neurologists and nurses were available in the building where the epilepsy monitoring unit was located. The room was equipped with a direct interphone connection to the epilepsy monitoring unit nurses' control room.

6.3.2 Electromyography

Motor evoked potentials were recorded bilaterally with surface electrodes positioned over the abductor pollicis brevis muscles, using a Nicolet Viking EDX electromyograph (Natus, Madison, WI, USA). Data were recorded with a sampling frequency of 4kHz and stored for offline analysis.

6.3.3 Electroencephalography

EEGs were recorded during the TMS sessions with a 64-channel TMS-compatible DC-EEG system (Waveguard^{TM} cap and ASAlab^{TM} software, ANT-neuro, Enschede, The Netherlands), a sampling frequency of 4kHz, and a common ground reference between the Fz and AFz electrodes. Participants were seated in a comfortable chair with their eyes open and arms in supine position. First, baseline EEG was recorded for 10 minutes with eyes open and closed.

6.4 Stimulation protocols

During TMS, soft earplugs were used to reduce discomfort from the coil click and lower the auditory evoked potential. The investigators ensured that the participants kept their eyes open during the experiment and monitored the EEG for signs of drowsiness.

6.4.1 Photic stimulation

After the baseline EEG recording, photic stimulation (Sigma, Is FSA 10-2D-I, SIGMA Medizin-Technil GmbH, Gelenau, Germany) was done according to clinical protocol, at 2, 6, 12, 20, 30, 40, 50 and 60 Hz with eyes closed and open (± 5 seconds each). If this elicited epileptic discharges in people with JME, stimulation was stopped immediately, then resumed at 60Hz and decreased until another discharge was seen, to determine

the range of frequencies to which an individual was sensitive. Photic stimulation was not performed in people with migraine.

6.4.2 Single-pulse stimulus response curve on Cz, estimation of rMT

The rMT was visually approximated with the coil on the vertex (electrode position Cz), starting at 20% stimulator output with 5% stepwise increments until a consistent twitch in the hand contralateral to the stimulated hemisphere was seen in 50% of the trials. In a great majority of cases, the muscle twitch was first seen in the abductor pollicis brevis muscle. Then, a semi-automated, in-house designed scanning protocol (created in Matlab® (version 7.5.0 R2007b The MathWorks Inc., Natick, MA, USA)) was used to deliver stimuli with an interstimulus interval of 2s. For the controls, the first measurement was done with 8 stimuli at each intensity, and the second with 20 stimuli. For the people with JME, between 8 and 20 stimuli were given at every intensity, and 20 stimuli in migraine. Scanning started at a stimulator output value of 10-12% below the visually approximated rMT and increased in 2% steps until a reproducible MEP (>200uV) was seen after every stimulus (± 110-120% rMT). The rMT was visually determined by the two investigators present during the measurement as the intensity at which a muscle twitch was seen in approximately 50% of the stimuli (MEP>50uV). The scanning procedure was performed using anti-clockwise (right hemisphere, Cz+) and clockwise (left hemisphere, Cz-) stimulation. The procedure was repeated with the placebo coil.

6.4.3 Paired pulse protocols

Paired pulse stimulation was done with the round convex coil on Cz. For the LICI recovery curves, two pulses were given at 110% of the stimulus intensity of the visually estimated rMT. Stimulation was repeated six times for each interstimulus interval. The stimulus pairs were given in a fixed, sequentially increasing order, with 25ms increments, starting at 50ms and ending at 400ms (14 intervals). The time between each stimulus pair (inter-trial interval) was one second. The unconditioned stimulus was given six times immediately before the start of the paired-pulse stimulation protocol. The recovery curves were constructed for each hemisphere. Paired-pulse protocols were performed in healthy controls and people with JME (with and without medication), but not in people with migraine.

6.5 Data analysis

Off-line analyses were done in Matlab® (release 2015, The MathWorks Inc., Natick, MA, USA). For each participant, TMS-EMG variables were computed for real stimulation (both polarities), and TMS-EEG variables were computed for the three TMS stimulation protocols (two polarities and sham stimulation). I used the sham stimulation to evaluate the effect of the auditory evoked potential from the coil click on the measures of phase clustering and non-linearity.

6.5.1 Calculation of the resting motor threshold

For each sequence of stimulation intensities (percentages of the maximal stimulator output) $A_k = \{A_1, \dots, A_n\}$, the corresponding MEP amplitudes $R_k = \{R_1, \dots, R_n\}$ were defined as the maximal peak-to-peak response recorded in both the EMG channels between 20-60ms after the TMS stimulus. The algorithm consists of two essential steps: data regularisation and threshold extraction.

Data regularisation was done to smoothen the stimulus-response curve variability within each measurement session. To determine the averaged EMG response amplitude for a set of test stimulus intensities $a_1 < a_2 \dots < a_M$, we applied non-polynomial Gaussian smoothening as follows:

$$G_u = \sum_k R_k e^{\frac{-(a_u - A_k)^2}{\sigma^2}} \Big/ \sum_k e^{\frac{-(a_u - A_k)^2}{\sigma^2}} \qquad (8)$$

We used a set of equidistant test intensities corresponding to the unique values of the sequence $\{A_1, \dots, A_n\}$ of pre-defined stimulator intensities. We define two values representing the motor threshold:

$$rMT1 \equiv maxarg\left(\frac{dG_u}{du}\right).$$
$$rMT2 \equiv maxarg\left(\frac{d^2G_u}{d^2u}\right). \qquad (9)$$

rMT1 represents the steepest point in the slope of the sigmoidal stimulus response curve (maximal value of first derivative). rMT2 detects the “taking-off” point of this

curve (maximal value of second derivative). For a sigmoid function rMT1 will therefore be larger than rMT2. An example of the analysis for one case is shown in figure 6.1.

6.5.2 Statistical analysis

The computed rMTs were compared to the visually estimated rMTs using the non-linear association coefficient h^2 described in **chapter 4** (Kalitzin *et al.*, 2007).

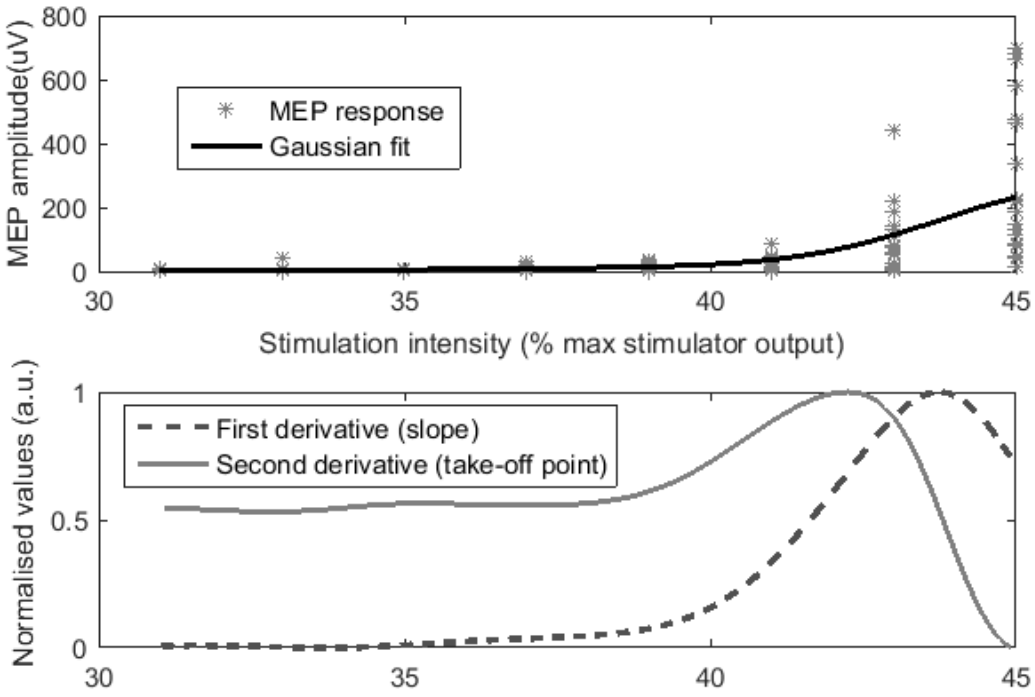

Figure 6.1: Motor threshold calculation based on the stimulation response curve. *Upper frame: A scatter plot of MEP responses (vertical axis in uV) versus stimulation intensity (horizontal axis in % of maximal stimulator output). Superimposed (black line) is the smoothened stimulus-response curve obtained by the Gaussian fit according to equation (8). Bottom frame: The first and second derivatives of the smoothened stimulus-response curve (dotted and solid curves respectively) as function of the stimulus intensity (horizontal axis). The two curves have been normalised in the [0 1] interval for better visualisation. The stimulation intensity at the maximal values of the curves corresponds to rMT1 and rMT2.*

6.5.3 Long-interval cortical recovery curves

To construct the LICI recovery curves, the mean MEP amplitude in response to the conditioned, second stimulus (test response) was divided by the mean MEP response to the unconditioned stimulus (conditioning response). For every group of participants, I calculated the median response ratio at every interstimulus interval, and the corresponding 25-75% interval. For comparison with the literature, the mean of the response ratio for each interstimulus interval was also computed, with the

corresponding standard error of the mean (i.e. the standard deviation divided by the square root of the number of participants).

6.5.4 Comparison with the literature

For comparison of our control group and JME group to the ones described in the literature, I made an overview of the group characteristics reported in the literature in different studies. I compared the LICI recovery curves across theses studies, by importing the .jpg files of the figures from the publications into Matlab®, using the standard Matlab routine "*imread*". I re-digitalised the curves, by defining the x- and y-axes and the positions of the data points of the curves relative to the x- and y-axes, so that the x- and y-coordinates of the data points could be estimated. The re-digitalisation procedure was repeated by independently by my colleague Annika de Goede.

6.5.5 EEG analysis: Phase clustering

Phase clustering analysis has been described previously (Kalitzin *et al.*, 2002; Parra *et al.*, 2003). The phase clustering index describes the phase consistency, with zero representing completely scattered phases and one maximal phase grouping. We computed the phase clustering index both after TMS, and photic stimulation for each participant, per EEG channel. Epochs of 100ms starting 8ms after stimulation were used. First, a discrete Fourier transformation was done on these epochs producing frequency bands of multiples of 10 Hz, from 10-500Hz (50 components). Then, the phase clustering index (PCI) was computed for each complex amplitude F obtained from the Fourier transform, frequency band n, stimulus number k and EEG channel α using equation (10).

$$PCI_{\alpha}^{n} = \frac{\sum_k F_{\alpha k}^{n}}{\sum_k |F_{\alpha k}^{n}|} \qquad (10)$$

The *relative* phase clustering index (rPCI), the maximal phase clustering index at a given frequency relative to the phase clustering index of the base frequency (10Hz), was then computed by:

$$rPCI_{\alpha} = max_n(|PCI_{\alpha}^{n}| - |PCI_{\alpha}^{1}|) \qquad (11)$$

Where $|z|$ is the magnitude (the absolute value) of a complex number z. The channel-averaged $rPCI_{tot} \equiv \langle rPCI_\alpha \rangle_\alpha$ was further used to classify the subjects. The channel- and subject group-averaged $PCI^n_{tot} \equiv \langle PCI^n_\alpha \rangle_\alpha$ per frequency was analysed across the frequency spectrum. To obtain the spatial distributions of the phase *P*, we calculated the group-averaged spatial distribution of the (complex) components of the highest phase clustering index per channel (equation (12)).

$$P_\alpha = PCI_\alpha^{nmax_\alpha}; nmax_\alpha \equiv argmax_n(|PCI^n_\alpha|) \quad (12)$$

Responses to photic stimulation frequencies of 2Hz, 6Hz, 10Hz, and 16Hz were analysed. The number of stimulations per frequency varied per participant. Both eyes open and eyes closed trials were included. For 2-10Hz, we used 100ms epochs. For 16Hz stimulation the epoch duration was 62.5ms. The channel- and subject-group averaged phase clustering index per frequency and the subject group mean relative phase clustering index for each channel were calculated for each stimulation frequency as described above.

6.5.6 EEG analysis: Non-linearity

For the calculation of the non-linearity, for each stimulus *k* and each channel α, the evoked EEG power was calculated using a window of 20ms with an offset of 3ms after the stimulus. The baseline EEG power was calculated using a window of 20ms up to 3ms before the stimulus. The response modulation $RM_{a,k}$ for each stimulus was defined as the logarithm of the ratio between the standard deviation of the evoked EEG power R_{evoked} and the standard deviation of the baseline EEG power $R_{baseline}$ and then averaged over all 64 EEG channels, α=1..64.

$$RM_{\alpha,k} = log\frac{R(\alpha,k)_{evoked}}{R(\alpha,k)_{baseline}}; \alpha = 1 \dots,64; k = 1..n \quad (13)$$

A Gaussian smoothing procedure was applied to reconstruct the stimulus response curve of the stimulation amplitude and *RM*. For each stimulation amplitude (% maximal stimulator output) $A_k = \{A_1, \dots, A_n\}$ and responses computed in equation (13): $RM_k = \{RM_1, \dots, RM_n\}$ and an aperture parameter σ, the Gaussian-averaged EEG response amplitude over the $a = 8$ or $a = 20$ stimulations $a_u = a_1 < a_2 \dots < a_M$ was computed using the non-polynomial smoothing procedure:

$$G_{\alpha,u} = \sum_k RM_{\alpha,k} e^{\frac{-(a_u - A_k)^2}{\sigma^2}} \Big/ \sum_k e^{\frac{-(a_u - A_k)^2}{\sigma^2}} \qquad (14)$$

The smoothed response function in equation (14) was then used to construct a stimulus response curve of the total EEG response against the stimulus intensity. The deviation of this fit after linear de-trending was calculated and is defined as "non-linearity". This analysis was only done for the TMS data, and not for photic stimulation, as it requires a change in stimulus intensity.

6.5.7 classification with relative phase clustering index and non-linearity

I used a linear classifier between relative phase clustering index of 0.4 and non-linearity of 0.4 to distinguish between people with epilepsy without medication and controls. The values of the linear classifier were chosen based on the data.

6.5.8 TMS evoked potential analysis

We also analysed the TMS evoked potentials (TEP), computed using epochs of 1s, starting 0.5s before the TMS-stimulus (4000 samples). The stimulation artefact was eliminated with linear interpolation from -10 to 10ms around the TMS-pulse. Epochs with EEG amplitudes >150uV were automatically rejected from analysis. Eye blinks were automatically rejected by selecting epochs with responses >80uV on electrodes FP1 and FP2. The data was baseline corrected, notch filtered at 50Hz and bandpass filtered between 1-80Hz. The TEP response was averaged for each electrode over all given trials. The group average was calculated as the mean of the responses on each individual electrode across individuals.

6.5.9 Statistical analyses

I quantified the relative phase clustering index after magnetic and photic stimulation and the non-linearity after magnetic stimulation in healthy controls, people with epilepsy with and without medication and participants with migraine. For comparison between the first and the second measurement in the healthy controls we used the non-parametric Wilcoxon-signed rank test at a significance level of 5%. The group averaged relative phase clustering index and non-linearity were compared between the migraine and epilepsy with and without medication groups and the second

measurement in the healthy controls using the non-parametric Kruskal-Wallis test (significance level 5%). The difference between clockwise, anti-clockwise and sham stimulation in each group was also compared using the Kruskal-Wallis test. I first performed phase clustering analyses without artefact rejection and re-analysed the data after rejection of epochs containing the 5% lowest and 95% highest responses, as well as with a different montage. There was no significant difference between the results with and without artefact rejection and with the second montage. I show the results obtained without artefact rejection, other than window selection. The window was optimised for phase clustering, non-linearity and TMS-evoked potential analyses individually and therefore varies.

7

Calculating the resting motor threshold & assessing cortical excitability with paired-pulse protocols.

"Far from humbling one's self before the great authorities of science, those beginning research must understand that – by a cruel but inevitable law – their destiny is to grow a little at the expense of the great one's reputation."[7]

In this chapter, I will describe the results obtained through Transcranial Magnetic Stimulation (TMS) and electromyography (TMS-EMG). First, I show how the resting motor threshold (rMT) can be calculated based on a stimulus-response curve. Then I present the long-interval cortical recovery curves that I measured in healthy controls and people with Juvenile Myoclonic Epilepsy (JME). I conclude this chapter by putting my findings in the context of some of the literature that I introduced in **chapter 2.2**.

7.1 Participants

7.1.1 Juvenile Myoclonic Epilepsy

Between May 2014 and October 2015, I included eight participants with JME (4 women, mean age 31.5 years, range 14-59). All were right handed according to the Edinburgh handedness questionnaire (Oldfield, 1971). Their characteristics are shown in table 7.1.

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[7] Ramón y Cajal S., (translation by Swanson N, Swanson LW). *Advice for a young investigator.* The MIT Press, Cambridge, MA, 1999, p11

Five participants were not using AEDs when they were included (case 1-5). Two people were photosensitive (cases 3 and 4). Two people started AED treatment and were re-measured six to eight weeks after treatment start (cases 3 and 5). The other three adopted lifestyle changes (case 1, 2 and 4). Three participants had been treated with AEDs for at least two years (case 6, 7, 8). Two of them did taper the dose (case 6 and 7) and were re-measured with a lower dose of AEDs. In total I measured five people without AEDs and five people with AEDs. All participants remained seizure free for the duration of the study (7-12 months).

Table 7.1: Characteristics of participants with JME.

Case Nr	*gender*	*age at inclusion*	*Photic Sensitivity*	*age at onset*	*handedness*	*Clinical features*	*medication first measurement*	*medication 2nd measurement*
1	F	14	N	14	9	TC, 1 febrile seizure characteristic pattern for JME on EEG	-	-
2	M	29	N	22	8	nocturnal TCs triggered by alcohol, characteristic pattern for JME on EEG	-	-
3	M	20	Y	20	9	nocturnal TCs triggered by alcohol, myoclonic jerks upon photic stimulation	-	levetiracetam 2d 500mg
4	F	34	Y	16	7	myoclonic jerks + TCs	-	-
5	M	17	N	15	9	myoclonic jerks + TCs	-	depakine 1d 600mg
6	F	59	N	16	9	myoclonic jerks + TCs + absences	depakine chrono 1d2000mg	-
7	M	24	N	14	8	myoclonic jerks + TCs	depakine 2d750mg	-
8	F	55	N	8	8	myoclonic jerks + TCs + absences	depakine 2d500	-

M=male, F=Female, N=no, Y=yes, TC=tonic-clonic seizures

7.1.2 Migraine

From June 2014 to October 2015, I also included twelve people with migraine (10 women, mean age 38 years; range 21-62). One participant was excluded because of beta-blocker use for high blood pressure. The characteristics of the participants with migraine are shown in table 7.2. The attack frequency was between 0.3 and 2 per month. Apart from one participant who habitually drank seven cups of coffee per day, daily coffee consumption in this group was limited. Three female participants were first-degree relatives. I analysed the results with and without two of these family members. Given the small differences between the two analyses, I report the results including the three family members. Experimental sessions were performed at least 72 hours after a migraine attack, and any measurements that were followed by a migraine attack within 72 hours were excluded.

7.1.3 Controls

I included 38 healthy participants between May 2014 and October 2014 (25 females, mean age 38.1 years range 15-62 years). One participant had to be excluded from the analyses because of non-specific EEG abnormalities. Five people were left handed (Oldfield, 1971). Thirty of the controls were re-measured after an average of 350 days (range 296-378 days) to assess the long-term reproducibility of the TMS-EEG measurements.

Table 7.2: Characteristics of participants with migraine with aura.

gender	age at inclusion	age at onset	handedness	diagnosis	attacks per month	% of attacks with aura
M	27	10	-9	migraine with aura	*u*	*u*
F	29	11	-5	migraine with aura	1	40
M	50	15	-7	migraine with aura	1	100
F	27	15	9	migraine with aura	0.3	90
F	21	19	9	migraine with aura	0.3	100
F	45	13	8	migraine with aura	1	100
F	35	22	8	migraine with aura	0.5	30
F	40	25	9	migraine with aura	2	100
F	62	17	-8	migraine with aura	0.5	100
F	51	18	9	migraine with aura	1	100
F	31	11	7	migraine with aura	1.5	35

u=unknown. Negative values indicate left-hand dominance.

7.2 Validation of motor threshold calculation in healthy controls

7.2.1 Correlation between visually estimated rMT and calculated rMT

All participants tolerated the experimental sessions well. None of them had a seizure or migraine attack following TMS or photic stimulation. To validate the calculated rMT1 and rMT2, from equations (8) and (9) with the visually estimated rMT, I used the first measurement in 37 controls, with 8 stimuli per intensity. The average acquisition time was 62.7 pulses for each hemisphere (125.4 sec). For both hemispheres, the rMT based on the visual estimation was highly correlated with threshold the calculated based on the first derivative of the stimulus response curve (h^2=0.898 for both left and right hemispheres p=0.002 and 0.001 for left respectively right hemisphere, see figure 7.1). The correlation between the visually determined rMT and the second derivative was slightly lower (left hemisphere: h^2=0.759, p<0.0001, right hemisphere h^2 =0.888, p<0.0001).

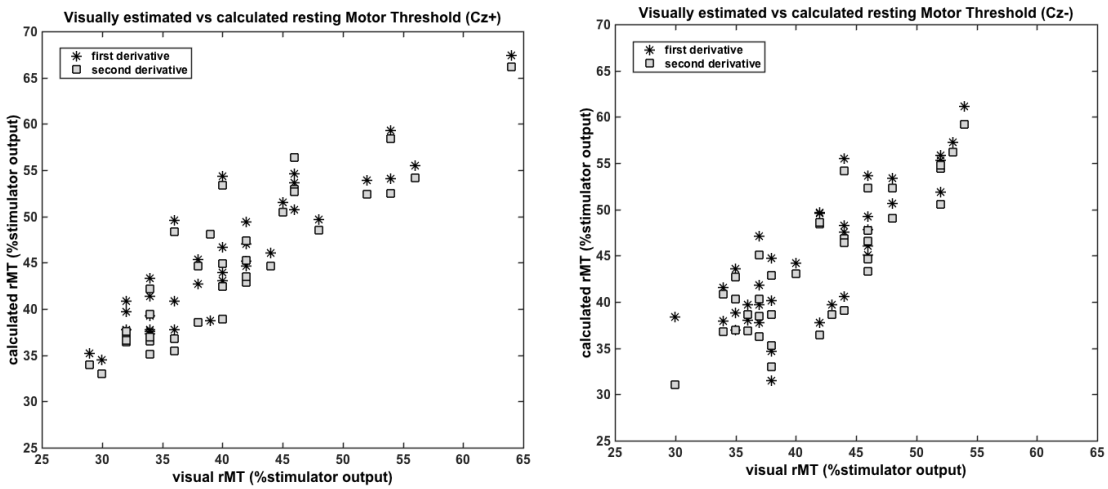


Figure 7.1: Correlation between the visually estimated threshold and computed thresholds. *The visually estimated rMT (horizontal axis) and calculated rMT (vertical axis) based on the first (stars) and second (squares) derivatives of the stimulation-response curve are shown (equation (9), page 122). Left panel: For anti-clockwise stimulation (Cz+, left hemisphere). Right panel: For clockwise stimulation (Cz-, right hemisphere). Note that the rMT based on the second derivative is mostly somewhat lower than rMT based on the first derivative.*

7.2.2 Comparison of the rMT between controls, people with JME, and people with migraine

The rMT determined using visual estimation for each group of participants is shown in figure 7.2. The rMT based on the calculations is shown in figure 7.3.

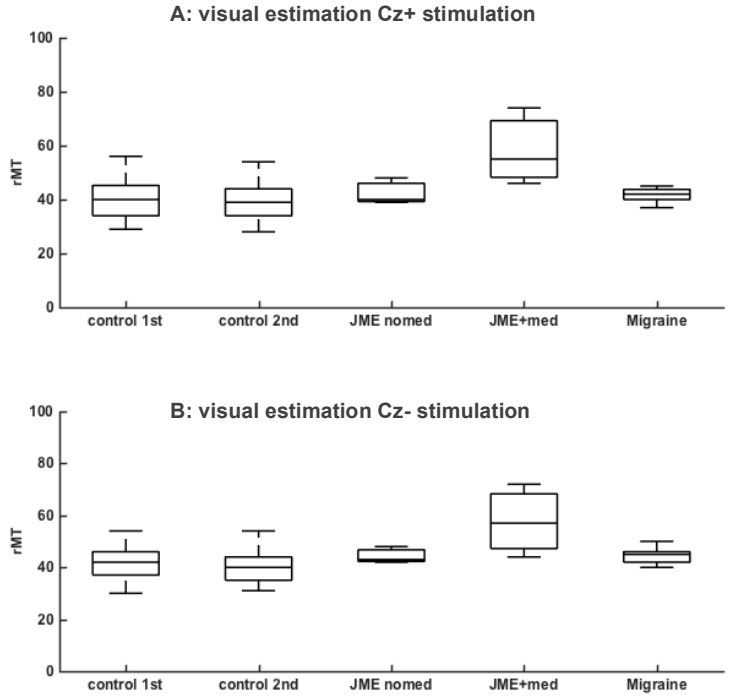


Figure 7.2: Visually estimated resting motor threshold for all groups. *Boxplots show the median and 25-75 percentile of the rMT for A: anti-clockwise stimulation (Cz+, right hemisphere) and B. clockwise stimulation (Cz-, left hemisphere). The rMT did not significantly differ between the groups.*

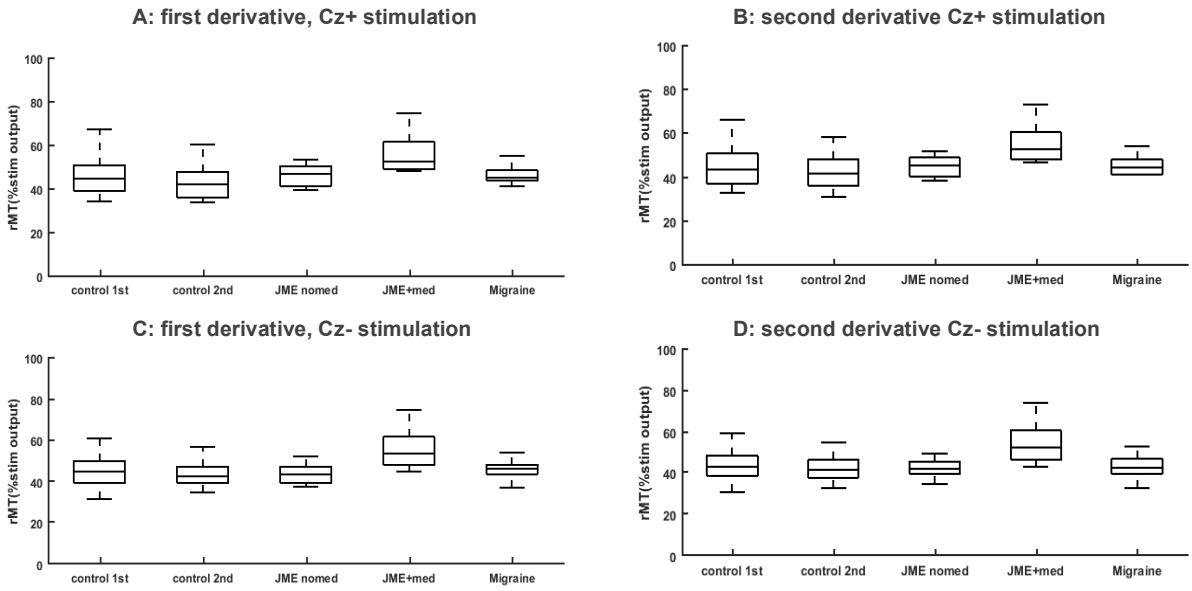


Figure 7.3: Calculated motor threshold for all groups. *Boxplots show the median and 25-75 percentile of the calculated rMT for anti-clockwise stimulation (Cz+, right hemisphere) A. based on the first derivative of the stimulus response curve, B based on the second derivative of the stimulus response curve. For clockwise stimulation (Cz-, left hemisphere) C based on the first derivative of the stimulus response curve and D based on the second derivative of the stimulus response curve The differences between the groups did not reach statistical significance.*

The first and the second measurements in the controls (8 and 20 stimuli per intensity) showed good reproducibility. There were no significant differences between the rMTs of the different groups, but there was a trend towards a higher rMT in the people with JME with medication compared to the other groups.

7.3 Paired-pulse protocols in healthy controls and people with JME

The paired pulse protocols were well tolerated by most participants. In one participant the protocol could only be completed for one hemisphere because the high motor threshold caused overheating of the coil. This protocol was done in healthy controls and people with JME, but not in people with migraine.

7.3.1 Long-interstimulus interval cortical recovery curves in healthy controls

The recovery curves for the first and second measurements in the controls are shown in figure 7.4. The ratio between the conditioned and unconditioned stimuli is shown on the vertical axis. A value between zero and one indicates inhibition, while a value above one indicates facilitation. At an interstimulus interval of 50ms, there is facilitation. Inhibition is visible around interstimulus intervals of 100-150ms. Around an interstimulus interval of 225ms there is facilitation in some people but not all. The reproducibility of the recovery curve is limited, and the inter-individual variability of the curve is high, as is shown by the single-case examples in figure 7.5.

7.3.1 Long-interval cortical recovery curves in people with JME

The long-interval recovery curves of the people with JME with and without medication (N=5) are shown in figure 7.6. There is a stronger facilitation at an interstimulus interval of 50ms than in the control group. With the anti-clockwise stimulation in people without medication, there is facilitation at interstimulus intervals longer than 300ms in some people, while the median indicates inhibition. In people with JME with medication, there seems to be more profound inhibition around 100ms than in people without medication. The facilitation around 50ms remains. The people with JME had a significantly higher facilitation than the controls at an interstimulus interval of 50ms ($p<0.05$). There was no difference between both groups at the other interstimulus intervals.

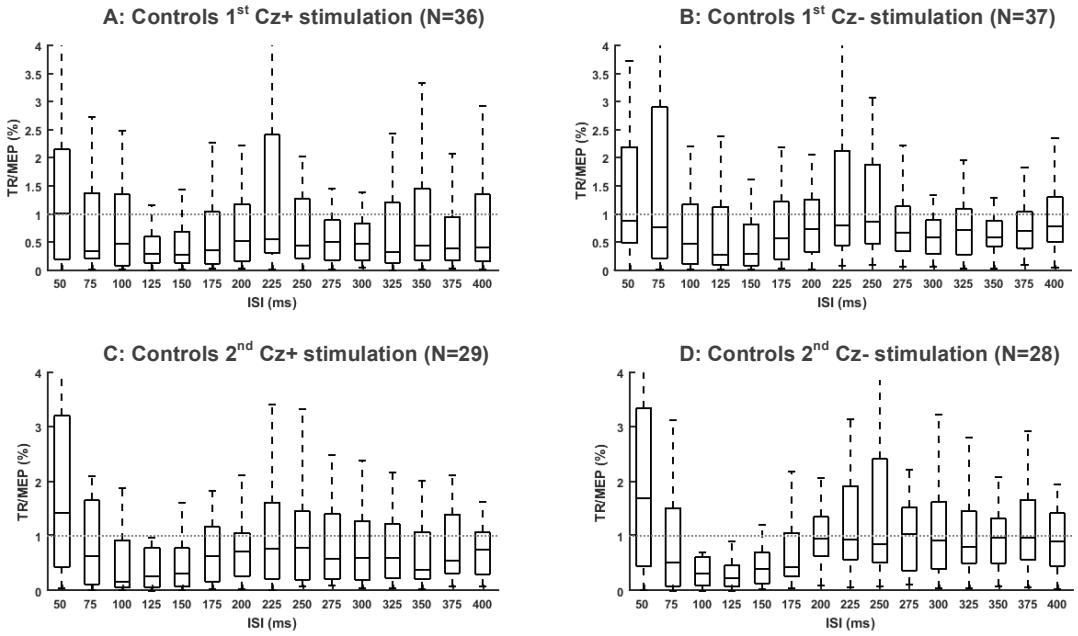


Figure 7.4: Long interstimulus interval intracortical inhibition (LICI) curve in controls. *Boxplots show the median and 25-75 percentile of the conditioned response for each interstimulus interval (ISI, in milliseconds on the horizontal axis) A: first measurement Cz+ stimulation (anti-clockwise, right hemisphere) B: first measurement, Cz- stimulation (clockwise, left hemisphere) C: second measurement Cz+ stimulation (anti-clockwise, right hemisphere) D: second measurement, Cz- stimulation (clockwise, left hemisphere). N= number of participants represented in each figure panel.*

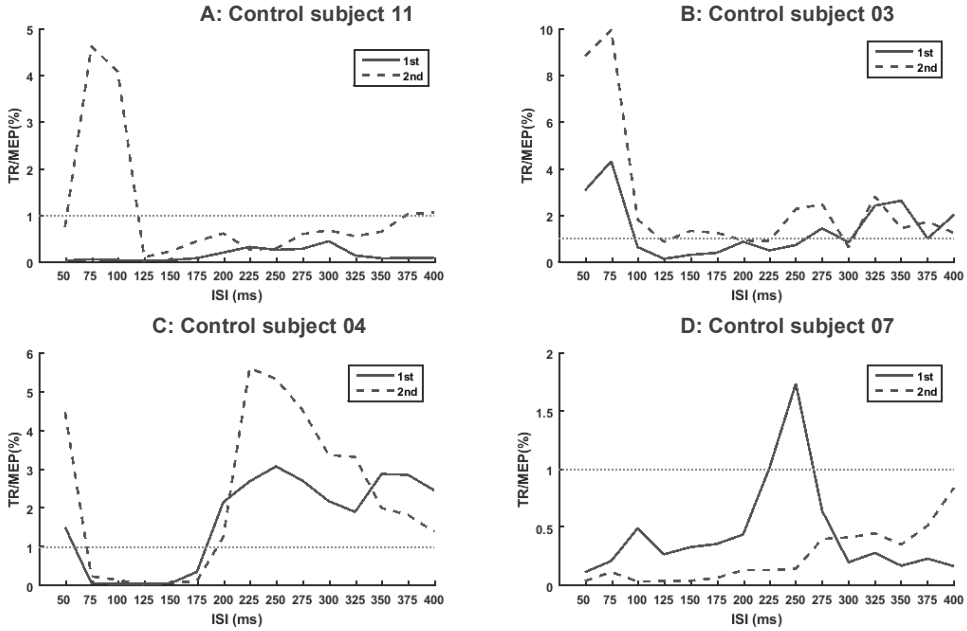


Figure 7.5: Long interstimulus interval intracortical inhibition (LICI) curve of four healthy controls. *The curves of four different controls after Cz+ stimulation (anti-clockwise, right hemisphere) of the first (solid line) and second measurement (dashed line) are shown. Horizontal axis: interstimulus interval in milliseconds. The vertical axes are different for each subject shown.*

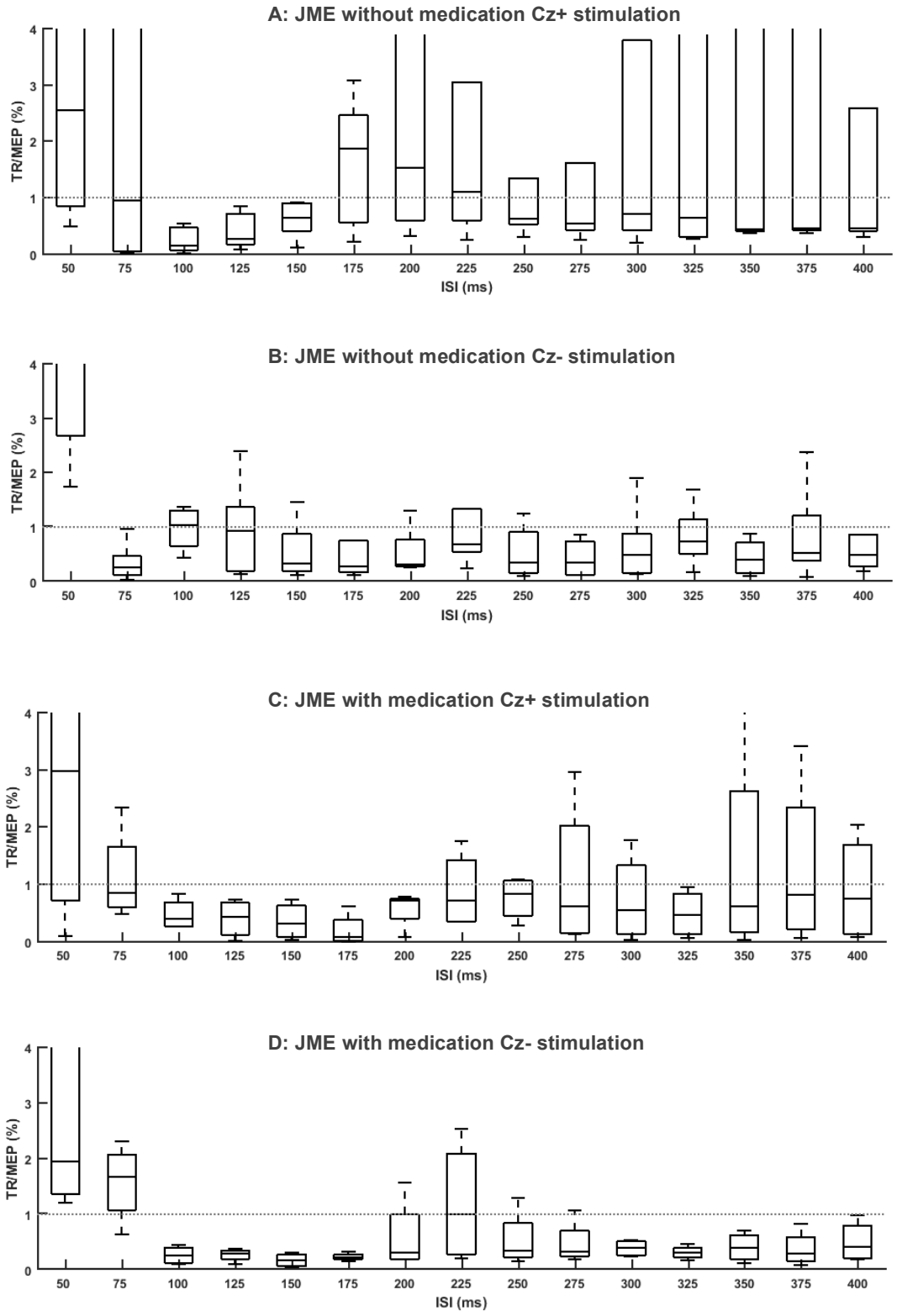


Figure 7.6: Long interstimulus interval intracortical inhibition (LICI) curves in people with JME. *A: anti-clockwise stimulation (Cz+, right hemisphere) in people with JME without medication B: clockwise stimulation (Cz-, left hemisphere) in people with JME without medication C: anti-clockwise stimulation (Cz+, right hemisphere) in people with JME with medication D: clockwise stimulation (Cz-, right hemisphere) in people with JME with medication. Boxplots show the median and 25-75 percentile of the conditioned response for each interstimulus interval (ISI, in milliseconds on the horizontal axis).*

7.4 Comparison with existing literature and critical re-appraisal

In healthy controls, long-interval cortical recovery curves with more than four interstimulus intervals were only reported previously by Valls-Solé *et al.*, 1992. The findings of this study are based on six healthy controls. The variability of their long interstimulus interval cortical recovery curve is similar to ours, see figure 7.7.

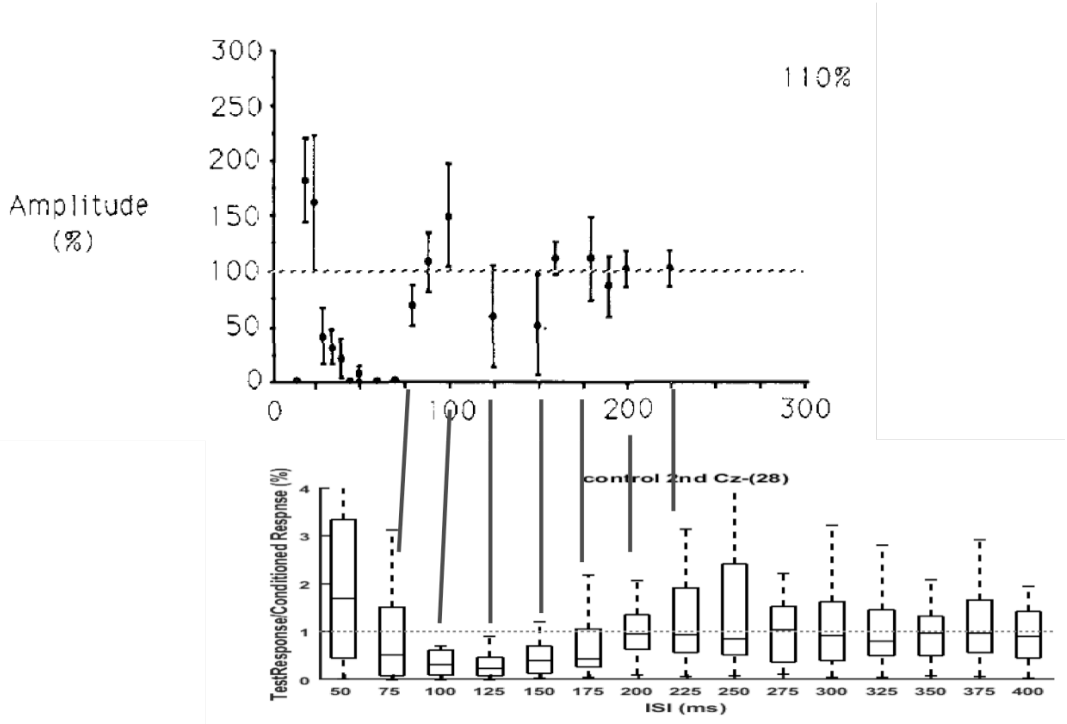


Figure 7.7: Comparison of my long interstimulus interval intracortical inhibition (LICI) curve with the literature. *Top frame shows the long interstimulus interval cortical recovery curve from 6 healthy controls published by Valls-Solé et al. (1992), for a stimulus intensity of 110% of the rMT (identical to the stimulation intensity I used). Bottom frame: my own long interstimulus interval cortical recovery curve from the second measurement on the left hemisphere (clockwise, Cz- stimulation), based on 28 healthy controls (see also figure 7.4D). The pattern is similar, except at an interstimulus interval of 100ms, where I found inhibition and Valls-Solé et al facilitation.*

Only the studies of Badawy *et al.* report long-interval cortical recovery curves with more than four interstimulus intervals in healthy controls and people with epilepsy, an overview of these studies can be found in **chapter 2.2**. In these studies, the inter-individual variability appears to be much smaller than in our groups. Badawy *et al.* also consistently report a difference between healthy controls and people with different types of epilepsy in several papers **(see chapter 2.2)**. Their curves show inhibition in controls at interstimulus intervals from 100-400ms. At 200ms there is usually neither facilitation nor inhibition (i.e. the ratio of the conditioned and unconditioned

response is one). The people with JME are reported to have facilitation at interstimulus intervals of between 150-250ms, and differ significantly from the controls at these interstimulus intervals (Badawy, Macdonell, *et al.*, 2009b; Badawy, Vogrin, *et al.*, 2013a, 2013c). In an attempt to understand why the variability in my sample is greater, I have examined the studies of Badawy *et al.* together with my colleague Annika de Goede.

7.4.1 Comparison with control groups in the literature

First, I examined the group characteristics of the studies. I found 18 studies reporting on TMS-EMG in healthy controls and people with epilepsy published by Badawy *et al* (**see chapter 2.2**, and table 7.3).

Table 7.3: Control group characteristics in studies of Badawy et al.

Article (journal, year)	*N*	*# of females*	*Mean age*	*Age range*	*rMT (mean±SD)*	*Subgroups*
1. Int J Neural Syst 2014	20	11	-	16-40	55.2 ± 5.6	
2. Clin Neurophysiol 2015	20	11	27	18-40	55.2 ± 5.6	
3. Epilepsia *a* 2013	20	11	27	18-40	55.2 ± 5.6	
4. Epilepsia *b* 2013	20	11	27	18-40	55.2 ± 5.6	
5. Epilepsia 2012	20	11	27	18-40	55.2 ± 5.2	
6. J Clin Neurophysiol 2012	19	13	20	16-28	55.2 ± 8.3	
7. Epilepsia 2010	32	20	31	16-73	56.9 ± 6.4	
8. Annals of Neurology 2010	32	20	31	16-73	56.2 ± 8.7	
9. Brain 2009	32	20	31	16-73	56.2 ± 8.7	
10. Int J Neural Syst 2013	30	19	28	16-61	56.2 ± 8.7	
11. Annals of Neurology 2013	11	11	23	18-40	53.9±5.4	Ovulatory follicular
					55.1±4.9	Ovulatory luteal
	9	9	24	18-40	54.1±4.9	Anovulatory follicular
					53.4±5.2	Anovulatory luteal
12. Epilepsy behav 2013	10	5	20	15-30	53.3 ± 5.1	fasting
					54.4 ± 5.3	Postprandial
13. Neurology 2009	10	6	29	21-46	55.1±7.1	morning
					56.4±6.7	afternoon
14. Brain 2013	12	7	-	-	55.4 ± 5.7	
15. Epilepsy Res 2012	17	11	30	23-50	56.1 ± 9.4	Session 1
					57.4 ± 7.9	Session 2
16. Clin Neurophys 2011	12	6	34	25-49	48.7 ± 7.2	Fig. 8, session 1
					48.8 ± 5.9	Fig. 8, session 2
					46.7 ± 5.3	Circular, session 1
					47.1 ± 7.1	Circular, session 2
17. Annals of Neurology 2007	29	12	33	13-73	57.1 ± 8.4	Dominant side
					46.8 ± 6.8	Non-dominant side
18. Neurology 2006	13	6	39.2	21-73	57.7 ± 5.7	Dominant side
					59.5 ± 7.2	After sleep deprivation

In eleven of these studies (number 1-11), the group characteristics are similar. From table 7.3, it appears that there are five studies (numbers 1, 2, 3, 4 and 5 from table 14) with 20 controls (11 women, mean age 27, age range 18-40 years). The reported mean rMT and standard deviation are exactly the same (except for the age range in study 1 and the standard deviation in study 5). Three other studies also have similar control groups (numbers 7, 8, 9 from table 14). All three have 32 controls, 20 of which were females, with a mean age of 31, and an age range of between 16-73 years. The mean rMT and standard deviation of study 8 and 9 is 56.2±8.7%, and of study 7 it is 56.9±6.4%. It is not reported in the papers whether these control groups are the same. If these are all different groups, then this means that the inter-individual variability of the rMT is low in their samples.

To try to understand the variability of the long interstimulus interval cortical recovery curve in these groups, I re-digitalised the published data and plotted them together in one figure. The re-digitalised curves from the publications 1-11 are shown in figure 7.8.

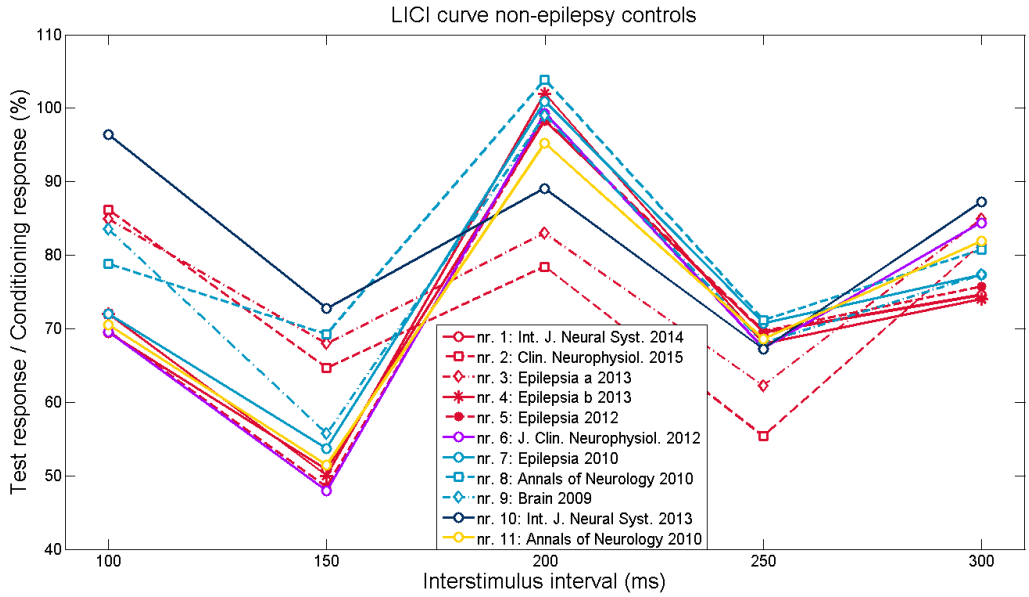


Figure 7.8: Re-digitalised long interstimulus interval intracortical inhibition (LICI) recovery curves of the controls from studies from Badawy et al. *This figure shows that the curves of studies 1 (red), 4 (red), 5 (red), 7 (light blue), 9 (light blue), 6 (purple), and 11 (yellow) are similar. The control groups of studies 1, 4, and 5 appear different from the control groups of studies 6, 7, 9 and 11, based on table 7.3. The curves of studies 2 and 3 (red) have the same pattern as the curve from study 10 (dark blue), while the control group characteristics are different.*

The long interstimulus interval cortical recovery curves show a "saw tooth" pattern that is seen in all the publications of this group. There is facilitation at the 50ms

interstimulus interval, consistent with the finding in my control group. In the results of Badawy *et al,* there generally is inhibition at the 150ms and 250ms interstimulus intervals in the controls. I did not see this pattern in my sample. In the studies of Badawy *et al*, error bars are shown in the figures of the long interstimulus interval cortical recovery curves, which I have not re-digitalised. It is not always described whether these represent standard deviations or standard errors. In cases where this is described, the error bars represent standard errors. Assuming that the control groups from studies 1, 2, 3, 4, 5 in the table consist of different individuals and based on the variability that I found in my own sample, I would expect that the pattern of the long interstimulus interval cortical recovery curves would vary, even if the control group characteristics are similar. From figure 7.8 it appears that the curves of studies 1, 4, and 5 overlap almost completely, and that the curves of study 2 and 3 show the same pattern. Interestingly, the curves of studies 6 and 11 also overlap with the curves of studies 1, 4 and 5, while the control group characteristics are completely different.

7.4.2 Comparison with JME groups in the literature

I also investigated the studies from Badawy *et al.*, which reported on a separate group with JME. The characteristics of the groups of people with JME and Juvenile Absence Epilepsy (JAE) from two of these studies are summarised in table 7.4. The numbers of the studies refer to the number in table 7.3.

Table 7.4: Generalised epilepsy group characteristics in studies of Badawy et al.

Article (journal, year)	# of controls	# of females	Mean age	Age range	rMT (mean±SD)
4. Epilepsia 2013					
JME new onset	10	6	20	14-23	49.3±7.9
JME refractory	16	9	25	15-40	53.6±5.1
JME seizure free	20	11	22	15-43	56.6±7.2
JAE new onset	8	5	18	14-23	54.5±5.7
JAE refractory	15	7	24	14-44	55.1±5.6
JAE seizure free	18	7	24	16-39	57.2±4.9
12. Brain 2013					
JME new onset	7	4	20	14-26	49.3±7.1
JME refractory	12	6	25	15-40	53.8±5.2
JME seizure free	14	6	22	15-43	56.4±7.1
JAE new onset	4	2	18	14-23	54.7±5.3
JAE refractory	12	7	24	14-44	55.3±5.5
JAE seizure free	12	7	24	16-39	56.9±4.8

JME= Juvenile Myoclonic Epilepsy, JAE= Juvenile Absence Epilepsy, rMT= resting motor threshold

The studies distinguish between people with JME or JAE with refractory seizures, people with well-controlled JME or JAE, and people with new-onset JME or JAE. It is not mentioned in the articles whether these groups of individuals overlap. The rMT of the same groups is similar in both studies, but not exactly the same. Again, I plotted the long interstimulus interval cortical recovery curves of these studies in the same figure (figure 7.9).

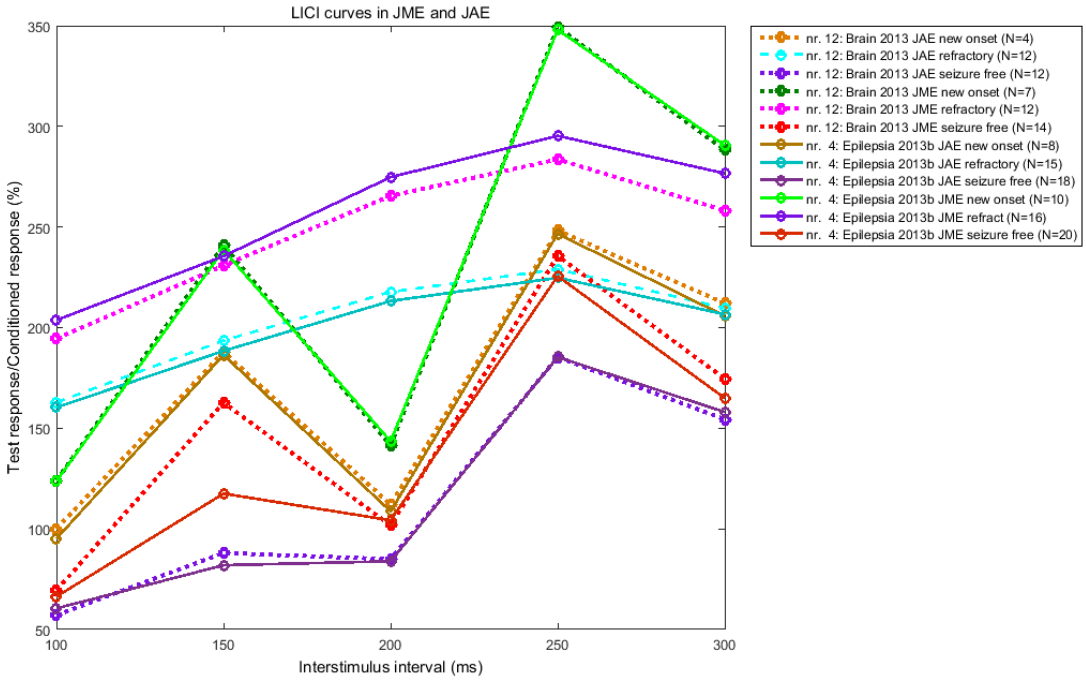


Figure 7.9: Re-digitalised long interstimulus interval intracortical inhibition (LICI) recovery curves of the JME and JAE groups from two studies from Badawy et al. *nr. 12 (dotted lines) and nr. 4 (solid lines). Based on the group characteristics and rMT from table 7.4, the curves belong to groups with similar age, sex and rMT characteristics, but with different sample sizes, indicated in brackets in the legend. The figure shows that despite different sample sizes, the LICI curves from similar groups from both studies overlap completely (new-onset JAE, brown lines, and new onset JME, green) or partially (JME seizure free, red).*

The curves show a pattern that is distinct from the healthy controls, with facilitation at between 150-250ms in most groups. At 200ms, the JAE seizure free group shows inhibition, the JME seizure free and new onset JAE groups show neither facilitation nor inhibition, contrary to the JME new-onset, JAE refractory, and JME refractory groups, which show facilitation. The curves from the different studies for the same groups seem to overlap for the new-onset JME group and the new-onset JAE group, despite the numbers included in the groups being different. I re-analysed my long

interstimulus interval cortical recovery curves for the controls and JME without medication, and instead of plotting the 25-75 percentile, I plotted the standard error of the mean (figure 7.10). For one of the stimulation polarities (Cz+, right hemisphere), the LICI curve of the JME group now shows a pattern similar to that described by Badawy *et al.*, with two facilitation peaks, one at an interstimulus interval of 200ms and one at an interstimulus interval of 350ms. In the controls, the curve shows neither facilitation nor inhibition at most interstimulus intervals.

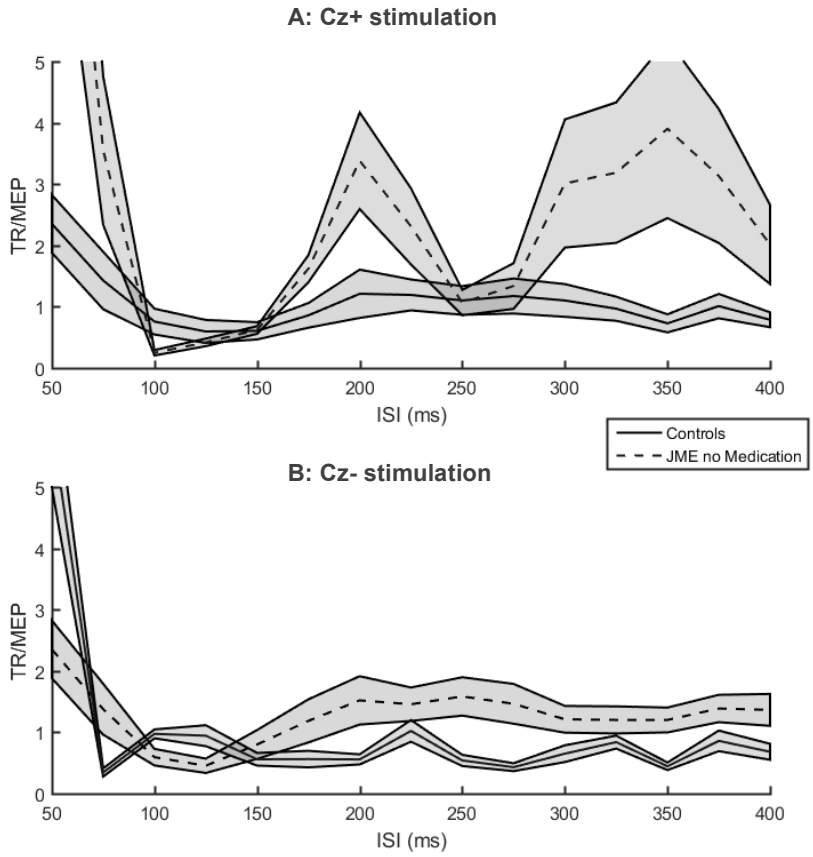


Figure 7.10: Long interstimulus interval intracortical inhibition (LICI) recovery curves with standard error of the mean. *The mean and standard error of the mean are shown (SEM). A: anti-clockwise stimulation (Cz+, right-hemisphere), B: clockwise stimulation (Cz-, left hemisphere). Compared to figure 7.4 and 7.6, the pattern of the curves looks more similar to the ones reported in the literature, and the inter-individual variability appears smaller.*

7.5 Discussion

7.5.1 Calculating the resting motor threshold

In the first part of this chapter I show that the rMT can be computed reliably by calculating the stimulus intensity at the maximum of the first and second derivative of the stimulus response curve. This method offers an objective yet simple way of extracting the rMT from the TMS-EMG stimulus response curve. The first and second measurements showed good reproducibility in the controls. In people with JME and people with migraine, the rMT was not significantly different from the controls.

Similar methods have been proposed previously, yet needed a relatively large number of stimuli (Mathias *et al.*, 2014; Tranulis *et al.*, 2006). It was recently reported that stimulus response curves can be acquired in a few minutes using a ramped design with interstimulus intervals as short as 1.4s (Mathias *et al.*, 2014; Pearce *et al.*, 2013). I have combined the insights of these studies to derive two measures of rMT from such a rapid stimulus response curve. It was previously shown that motor thresholds estimated using a figure-of-eight coil on the motor "hot spot", and a large circular coil on the vertex, did not significantly differ (Badawy *et al.*, 2011). To decrease the complexity of the procedure I chose to use a large circular coil, which, even when placed on the vertex, activated the motor cortex. This circumvents the need to locate the motor "hot spot" and makes the protocol easy to follow and transferable between investigators and institutions (Awiszus, 2014). I registered the EMG on the abductor pollicis brevis for the computation of the rMT. As I did not search for the exact motor hotspot, I used the previously described and validated "observation of movement" method for the visual assessment of the rMT (Varnava *et al.*, 2011). This method takes any movement from any arm muscle into account, and is therefore theoretically a better method to match with stimulation on the vertex than the standard method, which dictates that 50% of the trials should elicit a MEP of 50uV in the target muscle. Since I did not stimulate a specific muscle, this method may overestimate the motor threshold. My protocol, however, may also explain part of the variability in the rMTs measured and the difference between the rMTs of the left and right hemispheres. In my sample, stimulation on the vertex elicited a clear twitch in the abductor pollicis brevis in the vast majority of participants. One of the concerns with the proposed method may be that the rMT is overestimated. In the control group, the median rMT

is around 40% stimulator output, which is somewhat lower than described in the literature. This may be explained by the difference in stimulator, as the stimulator I used produces a stronger magnetic field than the commonly used Magstim 200, which is also used in the studies of Badawy *et al*. Whether the proposed methods overestimate the motor threshold needs to be addressed in future studies.

7.5.2 Long interstimulus interval cortical recovery curves

The long interstimulus interval cortical recovery curves that I measured in my sample are different from what is reported in the literature. In my sample, the long interstimulus interval cortical recovery curves did not follow a "saw tooth pattern" similar to those reported by Badawy *et al*. Comparison of the long interstimulus interval cortical recovery curves from different individuals in my sample, shows a large interindividual variability, consistent with some previous literature (Boroojerdi *et al.*, 2000; Cahn *et al.*, 2003; Du *et al.*, 2014; Valls-Solé *et al.*, 1992), but not in line with the work from Badawy *et al*. Stimulation of the right and left hemispheres in my sample yielded different results in the same individuals. This, again, may be caused by the different stimulators used. Our stimulator delivers biphasic pulses that lead to a tri-phasic magnetic current on the cortex. In the studies by Badawy *et al.*, a stimulator was used that delivers monophasic pulses, leading to a biphasic current in the brain. Another possible explanation for the different findings concerns differences between the stimulation protocols. I used fixed inter-trial intervals (between the pulse pairs) of 1s, while Badawy *et al.*, had a random inter-trial interval of at least 15s. They also delivered the different interstimulus intervals in a random fashion, while I gave the stimuli pairs in a fixed and increasing order. Lastly, I repeated every interstimulus interval six times, while Badawy *et al.,* repeated every interstimulus interval ten times. More repetitions per interstimulus interval may contribute to more robust results (Boroojerdi *et al.*, 2000). All these factors may have contributed to a difference between my findings and the findings reported by others previously, and need to be studied further. Another possible explanation for the difference is the presentation of the results. If Badawy *et al.,* do indeed show means and standard errors, this would explain why the inter-individual variability appears to be smaller in their studies than in my sample.

Some questions about the studies of Badawy *et al.,* remain. First, it is unclear whether the individuals in the control groups are the same in different studies. If, for example, controls were selected to achieve age matching with the patient groups, this would explain why the age and sex characteristics are the same in several studies. In view of the large inter-individual variability I found, there is a low probability that two groups consisting of completely different individuals have exactly the same mean rMT. It is also unlikely that groups consisting of different individuals would have such similar mean long interstimulus interval cortical recovery curves. The interpretation of the re-digitalised curves is limited by the re-digitalising procedure, which relies on the resolution of the published figures, and manually following the curves in the published plots. It is an estimation of overlap, rather than an exact calculation. It is clear that before paired-pulse TMS protocols can be implemented in a clinical setting for the measurement of cortical excitability, and these issues need to be addressed.

8

Measuring epileptogenicity with TMS-EEG

"To discover is to bring together two ideas that were previously unlinked."[8]

In this chapter, I describe how cortical excitability can be measured with TMS-EEG and single-pulse stimulation. I collected data from clockwise (Cz-) and anti-clockwise (Cz+) stimulation in controls (37 in the first measurement and 30 in the second measurement), in people with JME (five without medication and five with medication) and in 11 people with migraine. In addition, sham stimulation was done in controls (36 in the first measurement and 29 in the second measurement), in people with JME (four without medication and five with medication), and in 11 people with migraine. I used the sham stimulation to evaluate the effect of the auditory evoked potential from the coil click on the measures of phase clustering and non-linearity. Finally, photic stimulation was also performed in controls (35 in the first measurement and 29 in the second measurement), and in participants with JME (five without medication and five with medication), but not for the migraine group.

8.1 Phase clustering measured with TMS-EEG

The median relative phase clustering index for each group and stimulation modality is shown in table 8.1. The relative phase clustering index after magnetic stimulation did not differ significantly between the first and the second measurement in healthy

[8] Ramón y Cajal S., (translation by Swanson N, Swanson LW). *Advice for a young investigator.* The MIT Press, Cambridge, MA, 1999, p54

controls (p=0.504 for anti-clockwise stimulation and p=0.877 for clockwise stimulation). For statistical analyses, I used the second measurement for the controls, as the responses are averaged over stimulation intensity, the measurement with 20 pulses theoretically yields more reliable results. For sham stimulation in controls however, the relative phase clustering index was significantly larger in the first measurement than in the second. In the figures and boxplots, both measurements are shown. The relative phase clustering index after real stimulation was significantly larger in the JME group without medication than in controls (see figure 8.1 A and B). The relative phase clustering index in the JME with medication and the migraine groups did not differ significantly from controls. After sham stimulation, the relative phase clustering index differed significantly between the control, JME and migraine groups (see table 8.1 and figure 8.1C). The relative phase clustering index after photic stimulation at 6Hz was significantly larger in people with JME without medication than in controls (see figure 8.1D). There was no significant difference between the groups in the response to other photic stimulation frequencies.

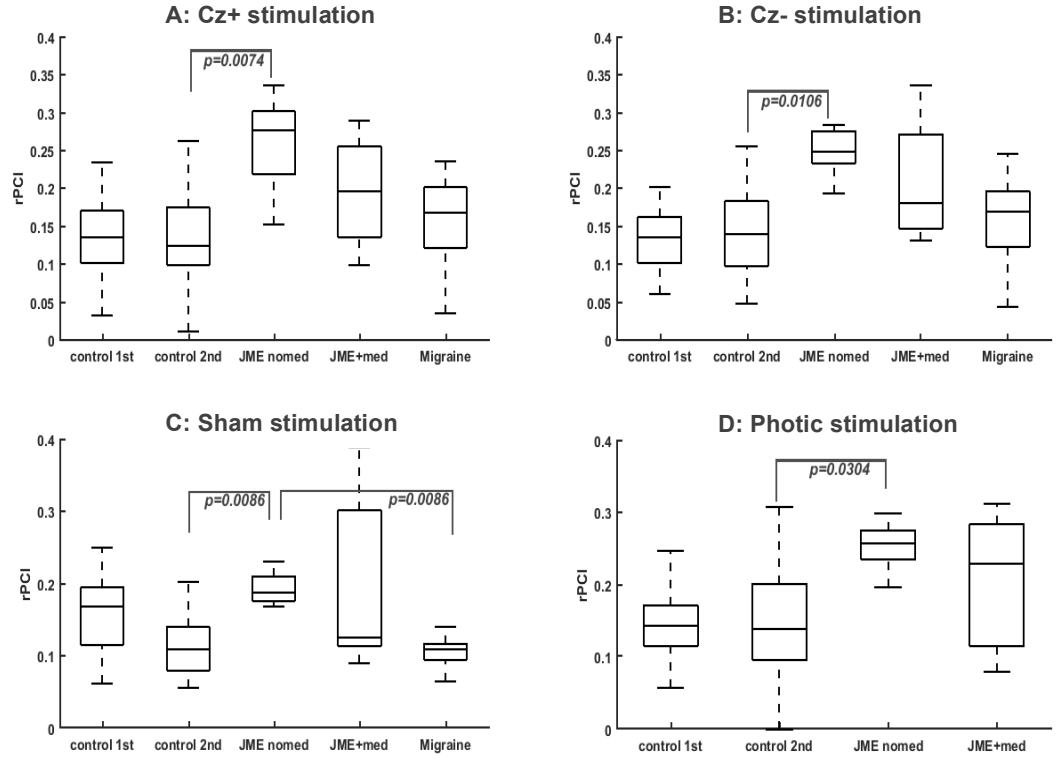


Figure 8.1: Boxplots of the relative phase clustering index (rPCI) for all groups. *The relative phase clustering index (rPCI, vertical axes, dimensionless) was averaged over all channels and is displayed per group. A. median rPCI after anti-clockwise stimulation (Cz+, right hemisphere) B. median rPCI after clockwise stimulation (Cz-, left hemisphere). C. median rPCI after sham stimulation. D. median rPCI after photic stimulation at a frequency of 6Hz. TMS frequency was 0.5Hz. The boxes show the 25 – 75th percentiles, the line in the box is the sample median.*

The boxplot in figure 8.2 shows the frequency at which the largest phase clustering index is found. For magnetic stimulation (0.5Hz), the phase clustering index in all groups was the highest in the gamma range (±40Hz). For photic stimulation at 60Hz, the maximal phase clustering index was found at around 60-80Hz (figure 8.2D). The phase clustering index after sham stimulation was variable and maximal at around 100Hz, and the largest for people with JME with medication (median 200Hz, figure 8.2C). There were no significant differences between the groups.

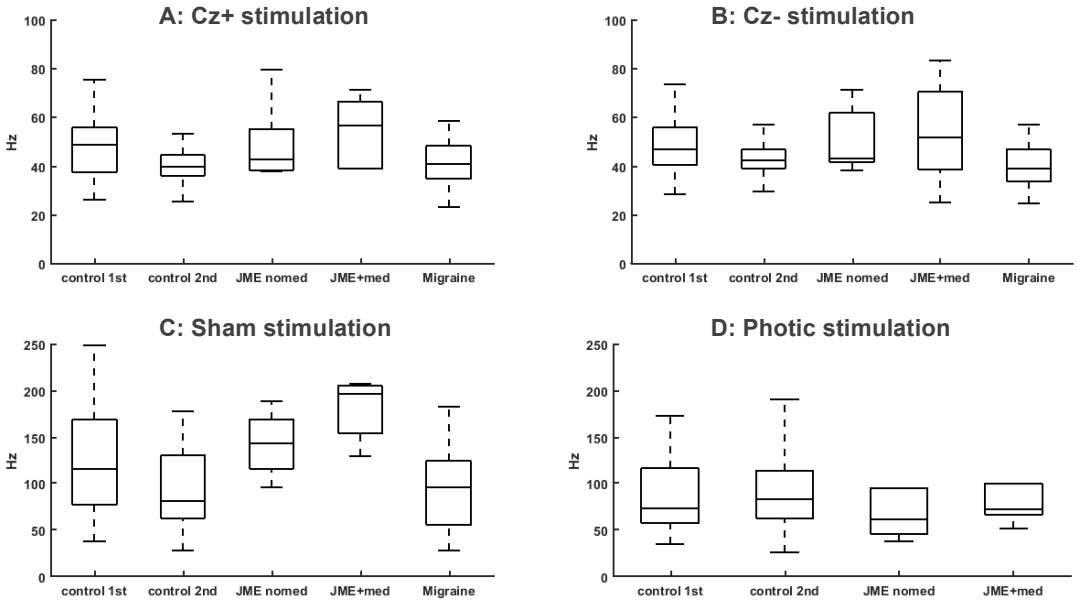


Figure 8.2: EEG frequency band of the maximal phase clustering index. *The EEG frequency band of the maximal phase clustering (PCI, vertical axis, in Hz) is shown for A. anti-clockwise stimulation (right hemisphere, Cz+) B. clockwise stimulation (left hemisphere, Cz-), C. Sham stimulation, and D. for photic stimulation at 6Hz. Magnetic stimulation frequency was 0.5Hz, with increasing stimulation intensity. The boxes show the 25 – 75th percentiles, the line in the box is the sample median. Note that the values of the y-axes for A and B are different from C and D.*

8.2 Non-linearity measured with TMS-EEG

The median non-linearity of the response is shown in table 8.2 and figure 8.3. There was no significant difference between the first measurement (8 stimuli per stimulus intensity) and the second measurement (20 stimuli per intensity) in the controls. The differences between the groups did not reach statistical significance (see figure 8.3A and B). In all groups, except for the JME with medication group, sham stimulation was significantly different from real stimulation (see figure 8.3C).

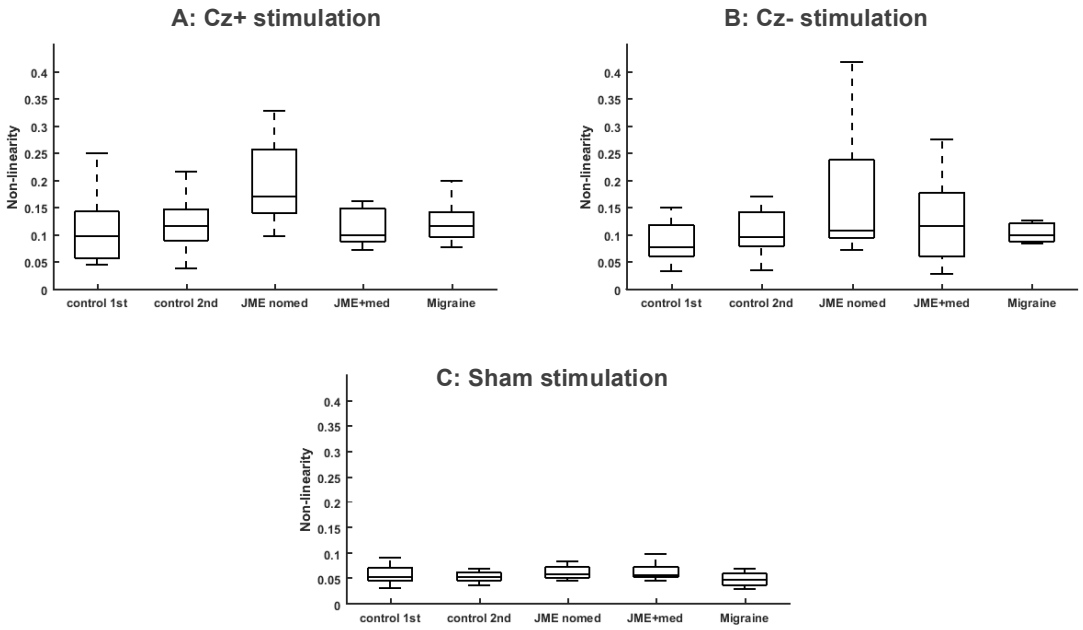


Figure 8.3: Boxplots of the non-linearity of the TMS-EEG response curve. *The median non-linearity of the TMS-EEG stimulus-response curve constructed using the Gaussian smoothing procedure described in equation 14 (page 126) averaged over all EEG channels and frequency bands is shown for A. anti-clockwise stimulation (Cz+, right hemisphere) B. clockwise stimulation (Cz-, left hemisphere) and C. Sham stimulation. The boxes show the 25 – 75th percentiles, the line in the box is the sample median. Non-linearity cannot be computed for photic stimulation as the analysis requires a change in stimulus intensity.*

Table 8.1: Median relative phase clustering index per group and stimulation modality.

	controls 1st	controls 2nd	JMEnoMed	JME Med	Migraine	KW between groups
Sham	0.1685 (0.0821 - 0.2389)	0.1095 (0.0593 - 0.1642)^	0.1878 (0.1686 - 0.2313)^	0.1264 (0.0906 - 0.3897)	0.1094 (0.0660 - 0.1401)^	p=0.0086
Cz+	0.1356 (0.0540 - 0.2866)	0.1244 (0.0777 - 0.2111)^	0.2766 (0.1526 - 0.3368)^	0.1959 (0.0983 - 0.2903)	0.1681 (0.0380 - 0.2348)	p=0.0074
Cz-	0.1363 (0.0655 - 0.2000)	0.1405 (0.0537 - 0.2552)^	0.2484 (0.1943 - 0.2841)^	0.1808 (0.1314 - 0.3358)	0.1690 (0.0471 - 0.2442)	p=0.0106
photic 6Hz	0.1468 (0.0625 - 0.2763)	0.1441 (0.0729 - 0.2794)	0.2575 (0.1969 - 0.2992)	0.2292 (0.0802 - 0.3127)		p=0.0304
KW within group (TMS)*	p=0.0464	p=0.0334 (sham vs Cz-)	p=0.1672	p=0.7788	p=0.0226 (sham vs Cz-)	

*The 5-95% are shown in brackets. KW=Kruskal-Wallis test. The KW test across groups is done using the 2nd measurement of the controls. * this p-value refers to the difference between sham, Cz+ (anti-clockwise, right hemisphere) and Cz- (clockwise, left hemisphere) stimulation. ^: significantly different groups in the (horizontal) group comparisons.*

Table 8.2: Median non-linearity per group for Transcranial Magnetic Stimulation.

	controls 1st	controls 2nd	JMEnoMed	JME Med	Migraine	KW between groups
Sham	0.0544 (0.0345 - 0.0839)	0.0536 (0.0423 - 0.1733)	0.0604 (0.0468 - 0.0850)	0.0578 (0.0463 - 0.0991)	0.0484 (0.0315 - 0.0697)	p=0.2782
Cz+	0.0988 (0.0481 - 0.2302)	0.1174 (0.0527 - 0.2160)	0.1705 (0.0976 - 0.3282)	0.0996 (0.0726 - 0.1626)	0.1166 (0.0780 - 0.1992)	p=0.2042
Cz-	0.0774 (0.0499 - 0.2429)	0.0966 (0.0441 - 0.1714)	0.1080 (0.0730 - 0.4176)	0.1170 (0.0293 - 0.2756)	0.0992 (0.0849 - 0.1830)	p=0.7906
KW within group	p<0.0001	p<0.0001	p=0.0308 (sham vs Cz-)	p=0.1013	p<0.0001	

The 5-95% are shown in brackets. KW=Kruskal-Wallis test.

8.3 Distinction of JME using non-linearity and phase clustering

The distinction of JME without medication, using a combination of relative phase clustering index and non-linearity, is shown in figure 8.4. For anti-clockwise stimulation (Cz+), all people with JME are to the right of the linear classifier. For clockwise stimulation (Cz-), all but two people with JME are to the right of the linear classifier. The people with JME with medication and people with migraine could not be differentiated from controls.

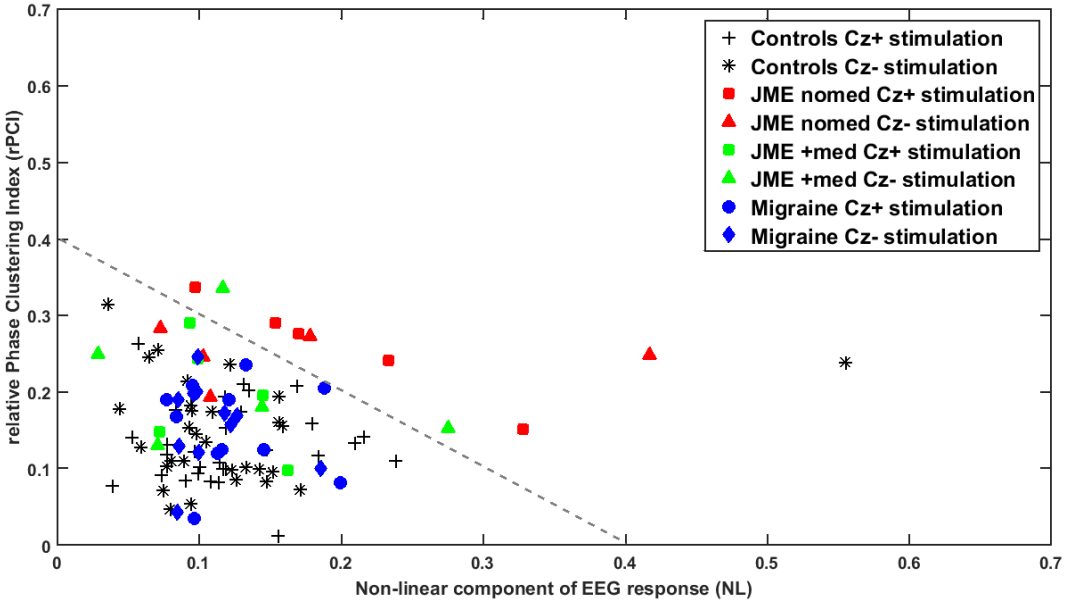


Figure 8.4: Scatter plot of non-linearity and relative phase clustering index. *For every individual in the sample, values obtained with anti-clockwise (Cz+, squares, crosses and circles) and clockwise stimulation (Cz-, stars, triangles and diamonds) are shown. For controls, the results of the second measurement (20 pulses per stimulation intensity) are shown. All but one of the subjects to the right of the linear separator are people with epilepsy without medication (red, N=5) or people with epilepsy with medication (green, N=2).*

8.4 Exploration of the topographical distributions of relative phase clustering index and non-linearity

Despite the study not being designed with the aim of localisation in mind, we investigated whether there are regional differences in relative phase clustering index and non-linearity. The topographical distribution of the relative phase clustering index is shown in figure 8.5. Both stimulation modalities elicit high relative phase clustering index in the frontal and occipital regions in the JME group compared to controls. The

topographical distribution of the relative phase clustering index, in response to photic stimulation, is similar to the pattern elicited with TMS.

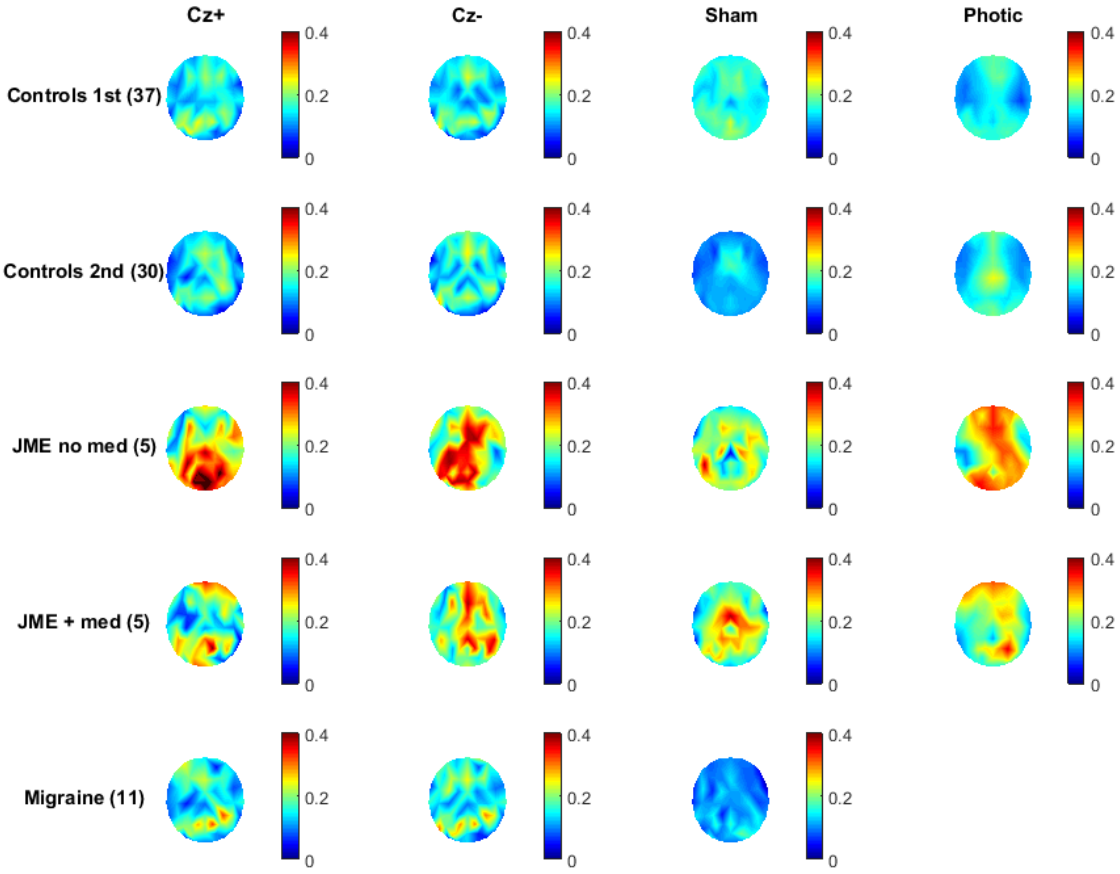


Figure 8.5: Topographical distribution of the relative phase clustering index. *Topographical rendering of the relative phase clustering index, calculated with equation (11) (chapter 6) for each EEG channel per group (rows) and per type of stimulation (columns, anti-clockwise (Cz+), clockwise (Cz-), sham, 6Hz photic stimulation). The number in brackets indicates the number of participants in the group that the measurement is based on. For sham stimulation in control groups and JME without medication the number of participants is N-1. Plot orientation: Fz at the top (north) and Pz at the bottom (south). Red indicates a high relative phase clustering index, corresponding to a high degree of phase synchrony, while blue indicates a low relative phase clustering index (i.e. scattered phases). The first (8 repetitions per stimulus intensity, top row) and second measurements (20 repetitions per stimulus intensity, second row) in the healthy controls yield similar results for anti-clockwise and clockwise stimulation. The relative phase clustering index is higher in the people with JME without medication (third row) than in controls in the occipital and frontal regions, especially after clockwise stimulation and photic stimulation. In people with JME with medication (fourth row), the relative phase clustering index is lower, but remains elevated compared to controls. In people with migraine (fifth row), the relative phase clustering index appears to be slightly elevated in the left occipital regions. A five-point pattern is visible in the healthy controls and people with migraine after anti-clockwise and clockwise magnetic stimulation, but not after photic stimulation or in the people with JME.*

An example of the relative phase clustering index change, following changes in the dose of levetiracetam in one participant with JME, is shown in figure 8.6. The relative phase clustering index decrease is inversely proportional to the dose of levertiracetam.

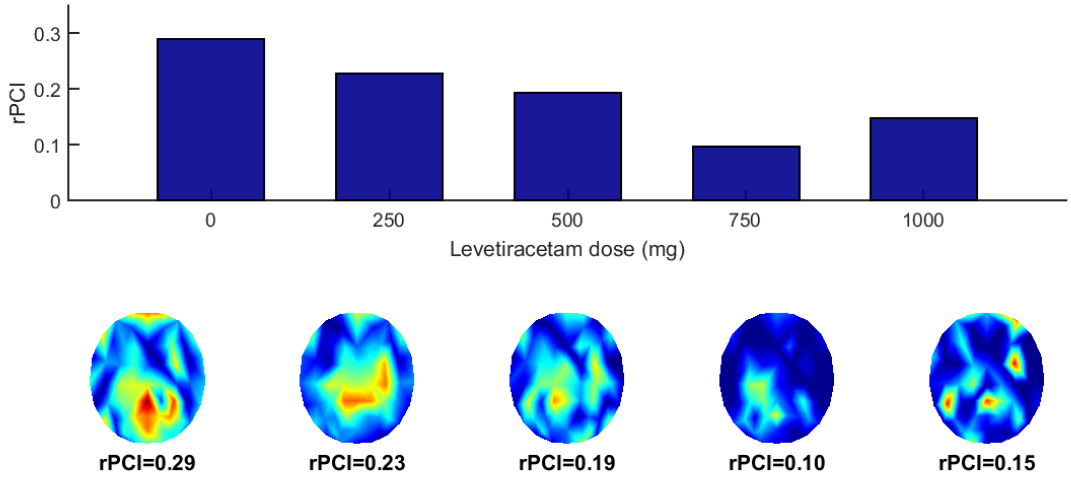


Figure 8.6: Effect of medication (levetiracetam) on the relative phase clustering index in one participant with JME. *For case 3 of table 7.1 the evolution of the relative phase clustering index and levetiracetam dose are depicted. This is the only participant in whom more than 3 measurements were done with different medication doses. The relative phase clustering index is shown on the y-axis and each dose of levetiracetam on the x-axis. The corresponding topographical renderings of the relative phase clustering index are shown below the barplot. The plots are not shown in chronological order, as this photosensitive participant started with 1000mg levetiracetam, but the dose was decreased to 250mg because of side-effects. Two measurements were done while he was taking 250mg levetiracetam, the average is shown in the figure. The participant remained seizure free for the duration of the study. During the last measurement (250mg), no photoparoxysmal reaction was seen, whereas this had been present during the other measurements.*

The topographical distribution of the non-linearity is shown in figure 8.7. Under sham stimulation there is larger non-linearity in the temporal regions in the JME groups (with and without medication), compared to controls and migraine groups. In the JME without medication group, non-linearity is higher in the frontal regions after real stimulation compared to controls.

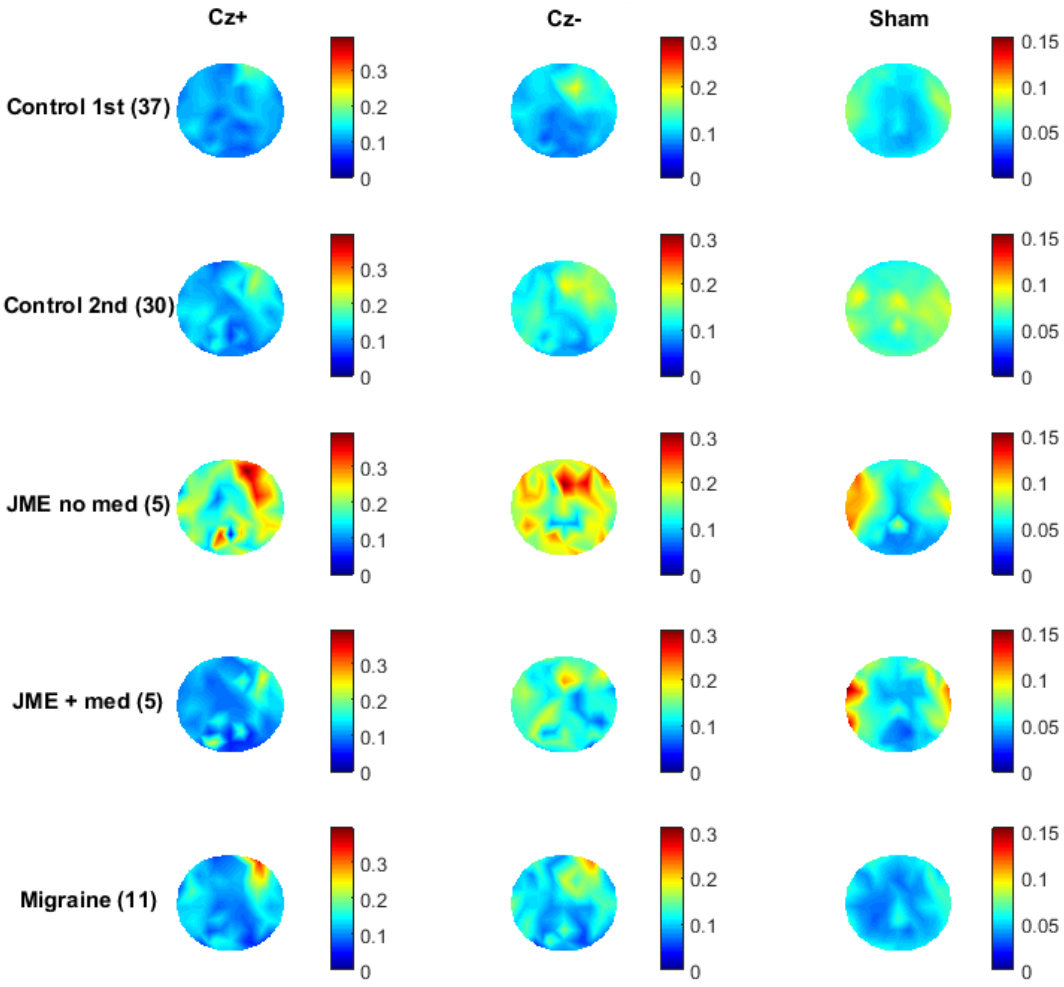


Figure 8.7: Topographical distribution of non-linearity of the TMS-EEG response curve. *Topographical rendering of the non-linearity, calculated with equation 14 (chapter 6), for each EEG channel per group (rows) and per type of stimulation (columns, anti-clockwise (Cz+), clockwise (Cz-), sham). The number in brackets indicates the number of participants in the group that the measurement is based on. For sham stimulation in control groups and JME without medication the number of participants is N-1. Plot orientation: Fz at the top (north) and Pz at the bottom (south). Red indicates a high non-linearity, corresponding to a higher deviation from a linear TMS-EEG stimulus-response curve, while blue indicates a low non-linearity (i.e. linear evolution of the response to the stimuli). The first (8 repetitions per stimulus intensity, top row) and second measurements (20 repetitions per stimulus intensity, second row) in the healthy controls yield similar results. The non-linearity is higher in the frontal regions in the people with JME without medication (third row) than in controls. This difference disappears almost entirely in the people with JME with medication (fourth row). In the people with JME both without and with medication, a small elevation of non-linearity is seen in the temporal regions after sham stimulation. Note that the scales of the colour maps are different for the three stimulation modalities (columns). The non-linearity cannot be computed for photic stimulation as it requires variable stimulation intensity.*

8.5 TMS evoked potential analysis

The TMS evoked potentials for a selection of channels for the controls, JME without medication, and migraine groups are shown in figure 8.8. Upon visual inspection, the waveform has a larger amplitude and displays more oscillations in JME. In migraine, the amplitude appears lower than in controls. In electrode Cz, the phase of the potential of the migraine group is shifted on the horizontal axis compared to the control and JME groups.

8.6 Spatial phase distribution

The phase difference seen in the evoked potentials was further investigated using the phase of the maximal phase clustering index (see equation (12) on page 125 in **chapter 6**). The results are shown in figure 8.9. In the JME without medication group, the phase is the same in the central and occipital regions, while in the migraine group, the phase in the central region is the same as the phase in the frontal region.

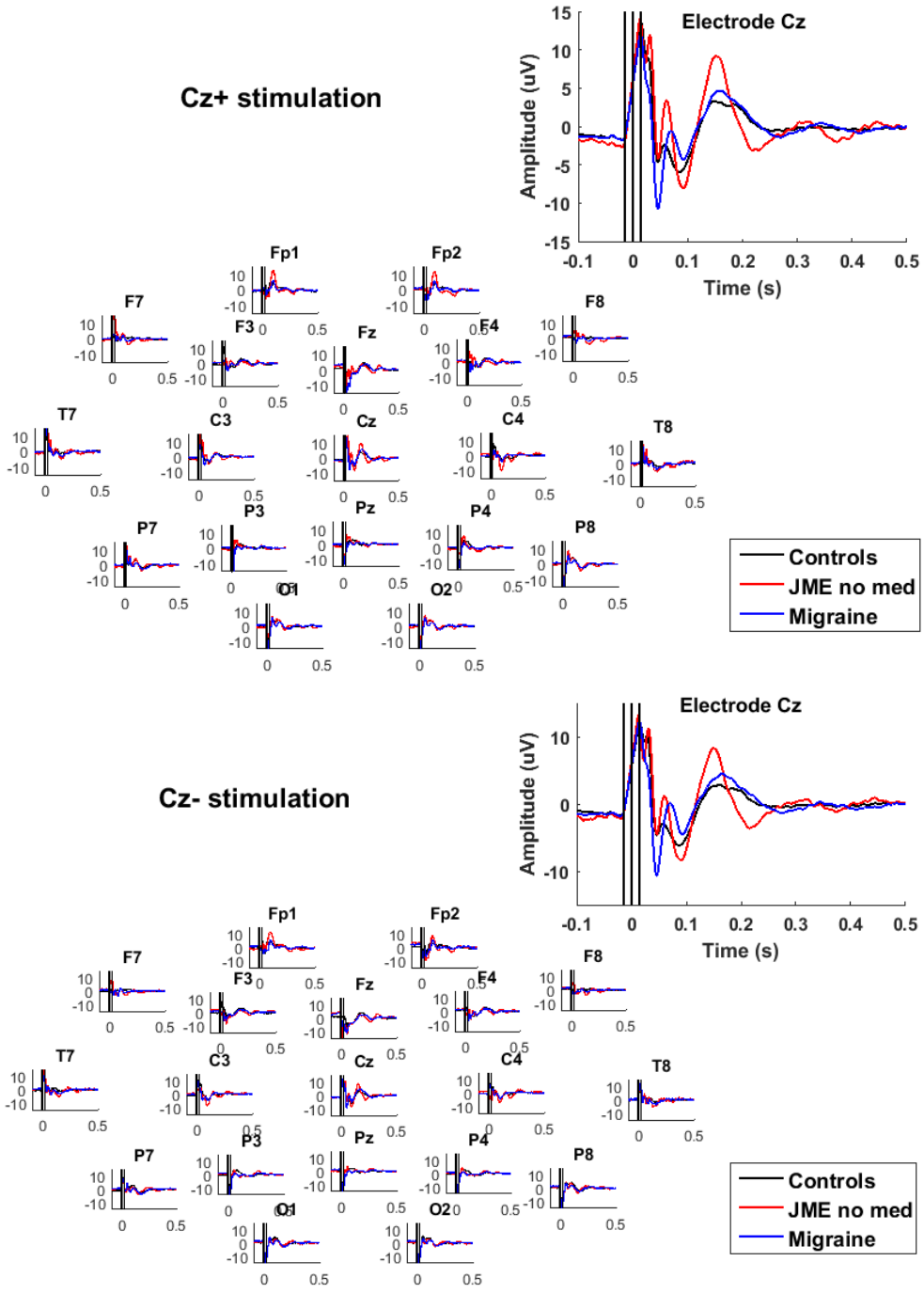
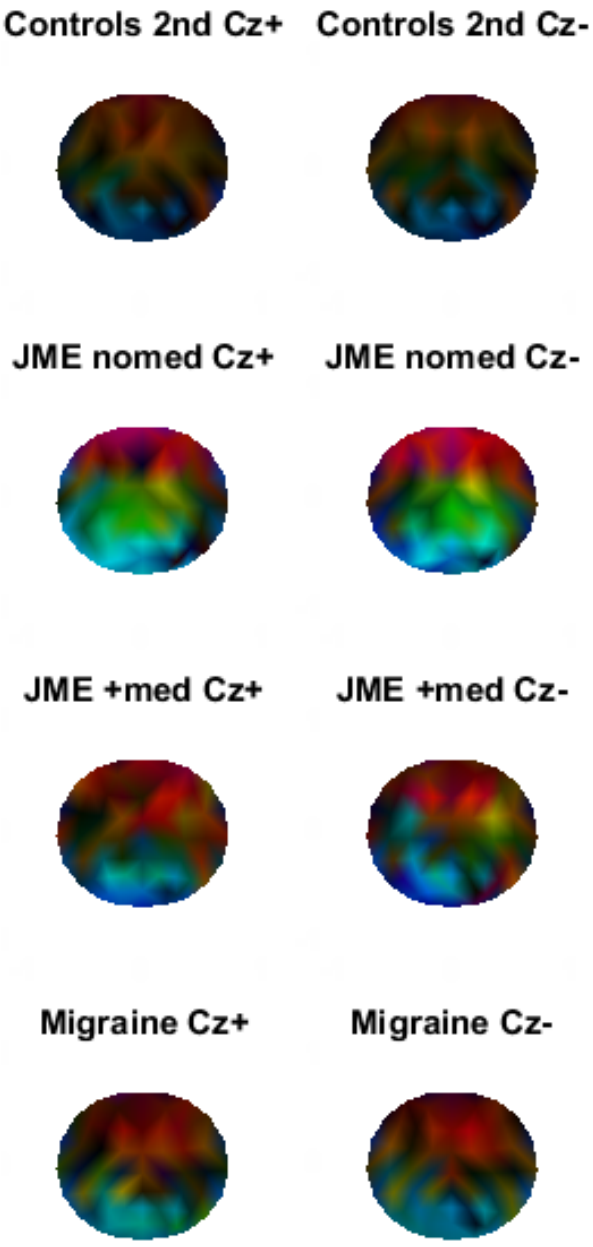


Figure 8.8: TMS-evoked potentials. *Averaged TMS evoked potentials for controls, JME without medication and migraine groups. For clarity purposes, 20 EEG channels are displayed, and the evoked potential on electrode Cz is shown in detail. The vertical lines around time point 0 indicate where the EEG signal has been intrapolated to correct for the TMS artefact (-10ms to 10ms).* ***Top frame:*** *anti-clockwise stimulation (Cz+, right hemisphere)* ***Bottom frame:*** *clockwise stimulation (Cz-, left hemisphere) For phase clustering analysis, epochs of 100ms, starting 8 ms after the stimulation were used, corresponding to the early phase of the evoked potential.*



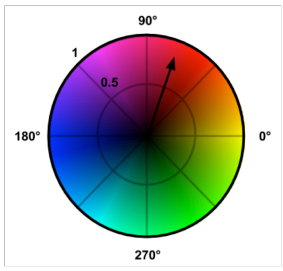


Figure 8.9: Group-averaged phase distribution of maximal phase clustering index. *Topographic distribution of the group-averaged maximal phase clustering index phase component (equation 12) for controls, JME and migraine groups for anti-clockwise and clockwise stimulation. The colour hue represents the phase of the maximal phase clustering index. The colour intensity represents the degree of clustering (the magnitude of the phase clustering index). The hue-phase and intensity-magnitude colour coding is presented in the plot showing the complex phase clustering index "disk" with phase clustering index=0 at the centre and phase clustering index=1 at the border. In the control group there is low phase clustering (dark colours). In the JME without medication group, the central regions have the same phase as the occipital regions (green). In the JME with medication and migraine groups, this is reversed, and the phase in the central regions is in line with the phase of the frontal regions (red).*

8.7 Discussion

In this chapter I have shown that the phase clustering, and the deviation of the linear TMS-EEG stimulus-response curve (non-linearity), can be measured using a TMS single-pulse paradigm. The phase clustering is elevated in people with JME compared to controls, and a combination of both non-linearity and relative phase clustering index differentiated people with JME from controls and people with migraine in my sample. In all groups, the largest phase clustering following photic and magnetic stimulation was found in the gamma range (40-80Hz), in line with previous reports (Parra *et al.*, 2003).

These findings support previous evidence of elevated phase clustering in magneto-encephalography recordings in people with photosensitive absence epilepsy in response to photic stimulation (Parra *et al.*, 2003). The larger relative phase clustering index in people with JME without medication may be indicative of increased propensity to synchronisation and entrainment of neural populations due to recurrent connectivity (Parra *et al.*, 2003). This increased tendency towards phase clustering, which is highest in EEG gamma band frequencies (40Hz), may be caused by the loss of a mechanism that inhibits excessive clustering such as GABA-ergic inhibition (Avoli and de Curtis, 2011; Wendling *et al.*, 2002). My colleagues and I hypothesise that increased recurrent connectivity, enabling synchronisation at these relatively high frequencies, may contribute to a larger phase clustering. In a recent study, increased phase clustering in the gamma frequency range in the EEG correlated with increased excitability in people with epilepsy (Meisel *et al.*, 2015). Despite the non-linearity not being statistically different between groups in my sample, when combined with the phase clustering index, it allowed reliable classification of people with JME on individual level. This indicates that phase clustering and non-linearity probably reflect necessary, but not sufficient, conditions for epileptogenicity (i.e. seizure proneness).

Our analyses were done without rigorous reduction of the artefacts caused by the magnetic stimulus, eye blinks, and muscle activity, and also without eliminating noisy EEG channels, as is often done for TMS evoked potential analysis (Ter Braack *et al.*, 2013; Hernandez-Pavon *et al.*, 2012). Re-analysis after rejection of epochs with outliers outside the 5-95% interval did not alter our results, nor did a different montage, suggesting that the relative phase clustering index is a robust variable. Comparison

with the results obtained using photic stimulation reveals a similar difference between people with JME without medication and controls, and a similar topographical pattern of the relative phase clustering index. This indicates that the phase clustering index is modality-independent, and probably represents a neuronal process rather than a measurement or muscle artefact.

The measurements for sham stimulation and real TMS were significantly different in the second measurement of the control group and in the migraine group, but not in the JME groups. This can be explained by the small sample size. Alternatively, the auditory stimulus of the coil may evoke a stronger response in people with JME than in the other groups, in line with the stronger response to visual stimuli. The question then arises as to whether magnetic stimulation is necessary at all, or whether any form of sensory stimulation may be enough to characterise such responses. Recent findings even suggest that phase clustering can be measured from on-going EEG without any form of stimulation (Meisel *et al.*, 2015). This is certainly an issue deserving further investigation, although the assessment of non-linearity always requires a stimulus-response curve, and thus a stimulus of variable strength. The analysis of TMS-evoked phase clustering and non-linearity can potentially be used for the localisation of areas with aberrant responses. The experimental set-up I used was not directed towards this goal. Firstly, I used global stimulation rather than focal stimulation, which would have been more suited for a study aiming at localisation. Secondly, I did not have the option to normalise topographical findings across individual subjects using imaging, which would have also enabled the definition of regions of interest prior to the experiment. Lastly, quantification of topographical differences of a variable measured using surface EEG requires source localisation procedures, which we did not use. My interpretation of the topographical renderings, however, suggests that the JME group has large phase clustering values in the occipital and frontal regions. I speculate that this could reflect increased connectivity throughout the brain, through which epileptiform discharges can quickly generalise. This would be in line with previous findings of altered connectivity between the occipital regions and supplementary motor area in people with JME which was discussed in **chapter 5.4** (Bartolini *et al.*, 2014; Vollmar *et al.*, 2012). In the migraine group, the relative phase clustering index was possibly slightly increased locally in the occipital regions. This may be in line with previous findings of increased excitability and defective inhibition in the visual cortex in migraine with

aura (Brighina *et al.*, 2015; Höffken *et al.*, 2009; Strigaro, Cerino, *et al.*, 2015). It was suggested that defective GABA-ergic mechanisms also play a role in migraine pathophysiology (Plummer *et al.*, 2011). A decrease in GABA levels was found in the occipital lobe in migraine with aura (Bridge *et al.*, 2015), corroborating with the suggestion of an excitatory-inhibition imbalance in this condition (Vecchia and Pietrobon, 2012).

In controls and people with migraine with aura, a five-point pattern linking the frontal, the parietal, and occipital lobes can be seen in the topographic relative phase clustering index plots obtained with real magnetic stimulation. It is not seen in the plots obtained with sham or photic stimulation, or in the non-linearity topographical plots. Based on the morphology of this pattern, and the fact that it is only seen after real magnetic stimulation, and in both control and migraine groups, I speculate that this is not an artefact and instead may reflect the spatial distribution of the main white-matter tracts involved in this process.

It is increasingly clear that the phase of neuronal oscillations offers valuable information (Lopes da Silva, 2006; Le Van Quyen and Bragin, 2007). I suggest that the phase difference observed in people with JME and people with migraine in the central region may be caused by differences in thalamocortical synchronisation. In migraine, thalamocortical activity underlying sensory processing was hypothesised to be reduced based on a decrease in somatosensory evoked high-frequency oscillations (Coppola *et al.*, 2005). Under visual stimulation, evoked gamma band activity appeared to increase in people with migraine with and without aura (Coppola *et al.*, 2007). Phase synchronisation in the beta band was decreased in people with migraine, possibly reflecting altered resonance of thalamic activity (de Tommaso *et al.*, 2013). The differentiated occipital activity, compared to central and frontal regions that I found in people with migraine with aura, may thus be driven by direct links between thalamic neurons and the visual cortex (Noseda and Burstein, 2013). Defective thalamic inhibition was previously linked to epileptogenesis (Sohal and Huguenard, 2003). In JME, structural abnormalities (Keller *et al.*, 2011; Pulsipher *et al.*, 2009) and reduced GABA concentration were found in the thalamus (Hattingen *et al.*, 2014). Speculatively, in JME, decreased thalamic inhibition increases the propensity to

hypersynchronisation, while increased thalamic inhibition in migraine with aura may lead to a propensity to desynchronisation (Hall *et al.*, 2004).

The study described in this chapter is limited by the small sample size, especially in the JME group. The stimulation protocol has to be further optimised for the analysis of the phase clustering and non-linearity. In a design with focal stimulation guided by imaging, the phase clustering index and non-linearity may potentially help localise cortical areas with aberrant inhibition. This may be particularly helpful in focal epilepsy, where surgical resection of the epileptogenic zone can lead to seizure freedom. Such a design was previously successful in localising cortical areas connected to subcortical heterotopic grey matter in periventricular nodular heterotopia using the TMS-evoked potential (Shafi *et al.*, 2015). Cortical excitability probably changes during the epilepsy and migraine cycle (Badawy, Macdonell, *et al.*, 2009a; Cosentino *et al.*, 2014; Delvaux *et al.*, 2001). Phase clustering was shown to increase when photic stimulation was followed by an epileptic discharge (Parra *et al.*, 2003). To improve the understanding of the clinical significance of phase clustering and non-linearity as TMS-EEG variables, further studies will need to assess their change before and after the ictal period. Another important issue is whether these variables could differentiate responders to anti-epileptic therapy from non-responders.

In conclusion, I show that TMS-EEG measures of clustering and multi-stability are potential markers of epileptogenicity in people with JME. These variables may contribute to the understanding of pathophysiological mechanisms in epilepsy and migraine, and may, in the future, have a direct clinical application in differentiating responders from non-responders and localising areas of increased excitability.

9

Summary, conclusions and context

"Each problem solved stimulates an infinite number of new questions, and today's discovery contains the seed of tomorrow's."[9]

9.1 Summary

In this thesis, I have shown that epilepsy, a paroxysmal neurological condition, shares features with migraine (**chapter 2**), and that the transitions from a normal to a pathological brain state may occur because of altered excitability. In **chapter 2.2**, I have provided a comprehensive literature review of cortical excitability, and experiments done with Transcranial Magnetic Stimulation (TMS) in people with epilepsy**,** which showed that cortical excitability is primarily dynamic, but generally elevated in people with epilepsy. From this review, I took the notion that TMS may be a promising technique to measure cortical excitability in clinical and therapeutical settings, despite the remaining questions regarding its predictive power on individual level. In **chapter 3,** I presented a meta-analysis on the co-occurrence of epilepsy and migraine. This study included more than 1.5 million subjects, and revealed a significant bi-directional association between migraine and epilepsy, supporting the hypothesis of shared, or overlapping, pathophysiological mechanisms.

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[9] Ramón y Cajal S., (translation by Swanson N, Swanson LW). *Advice for a young investigator.* The MIT Press, Cambridge, MA, 1999, p71

To further study the intriguing transitions between brain states in epilepsy, and especially postictal generalised EEG suppression (PGES), I tested hypotheses derived from a computational model in clinical video-EEG recordings in the study described in **chapter 4**. This interdisciplinary approach provided the interesting insight that seizure termination is not a random transition, and that the decrease of clonic frequency at the end of a generalised seizure is correlated with the duration of PGES. This study also demonstrates how simplified abstract computational models, combined and validated with clinical data, can be powerful tools to study complex mechanisms.

Chapter 5 described a straightforward clinical assessment of the regional distribution of interictal spikes in people with photosensitive JME (JME+PPR), and people with JME-PPR. The fact that focal abnormalities are less prevalent in the occipital lobe in people with JME-PPR than in people with JME+PPR is in accordance with studies showing a higher excitability of the occipital lobe in JME+PPR. It supports existing evidence that JME is a condition of altered brain networks.

The subsequent three chapters try to answer several questions that were raised in the first half of this thesis. Can the susceptibility to transition from a normal to a pathological, synchronous brain state in epilepsy be quantified using TMS-EEG? Can TMS-EEG reveal more about the supposedly shared pathophysiological mechanisms in epilepsy and migraine? Could TMS be implemented in the clinical practice to measure cortical excitability as a biomarker of disease activity in epilepsy? The methods for the study designed to answer these questions were outlined in **chapter 6.** In **chapter 7,** I showed how the resting motor threshold can be estimated automatically in about 2 minutes using a stimulus-response curve paradigm. Such a method can easily be transferred to a clinical setting and enables an objective calculation of this variable. The results from the paired pulse paradigm showed a large interindividual variability. I compared my findings with the existing literature (reviewed in **chapter 2.2**) and discussed differences and similarities. I concluded that several issues need to be resolved before paired-pulse paradigms can be used as a biomarker of disease activity in epilepsy in a clinical setting.

In **chapter 8**, I described how phase clustering, a measure of synchronisability, can be assessed using TMS-EEG or photic stimulation, and showed that it is elevated in people with JME, and possibly in the occipital lobe of people with migraine with visual aura, compared to controls. This method was stable across stimulation modalities, and in different approaches of signal pre-processing such as artefact reduction and montage. This makes phase clustering an attractive variable to study further. Combined with non-linearity, which may be a sign of bi-stability, it classified people with JME from healthy controls and people with migraine.
The findings of this thesis are summarised in figure 9.1.

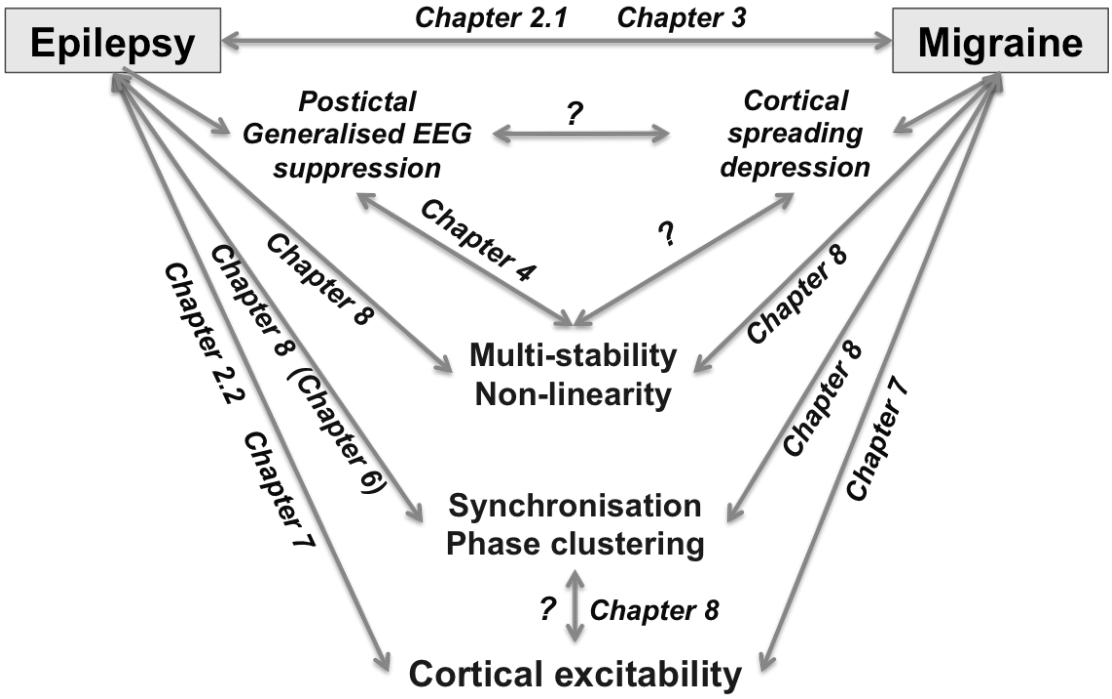

Figure 9.1: Overview of the findings of this thesis.

9.2 Applicability of the findings

Some of the insights from the studies in this thesis can be applied directly in clinical practice. The evidence for the co-occurrence of epilepsy and migraine encourages physicians to actively inquire about the presence of co-morbid symptoms. This enables the initiation of adequate therapy, and can potentially reduce the disease burden and associated costs (both direct and indirect). The same holds true for the finding of focal EEG abnormalities with occipital dominance in people with JME+PPR. The presence of

focal EEG abnormalities can make the diagnosis of JME challenging, and encourages physicians to obtain exact descriptions of the symptoms, including subtle myoclonic jerks, absences, and generalised tonic clonic seizures, and also less commonly described signs such as visual aura or conscious head version. Only with a detailed history, supported by an EEG recording, can adequate treatment be started. If JME is wrongly diagnosed as focal epilepsy, and subsequently treated with anti-epileptic drugs (AEDs) such as carbamazepine, this can have devastating consequences as it aggravates seizures in JME.

The novel and surprising finding that clonic slowing at the end of a seizure is related to the duration of postictal generalised EEG suppression may be used, if replicated, to develop a warning system. Postictal generalised EEG suppression was previously linked to sudden unexplained death in epilepsy, and a warning system may help prevent such fatal events by prompting interventions in the postictal phase, until more is understood about the pathophysiological mechanisms that cause sudden unexpected death in epilepsy and allow for more targeted prevention strategies. This study is also an example of "predictive modelling", the use of models to generate testable hypotheses. This strategy may be extremely valuable for phenomena that are difficult to study because they are rare (such as sudden unexpected death in epilepsy), or to disentangle different factors involved in complex processes. The other methodological insight from this thesis is the new approach to quantify TMS-EEG signals, using the phase clustering index and non-linearity. These may be promising variables for the study of localised changes in cortical excitability with TMS-EEG.

9.3 Limitations

The limitations of each study were discussed in the corresponding chapters. As is common in research, the greatest limitations of the studies in this thesis were a lack of time and resources to be able to answer the research question from all angles. At the very start of my PhD project in 2012, I set out with the goal of investigating whether TMS could be used to individually assess changes in cortical excitability in people with epilepsy, based on the promising evidence from the existing literature, especially the studies from Badawy *et al*. It seemed useful to limit this endeavour to one type of epilepsy in order to have a relatively homogenic group. JME was chosen for pragmatic reasons, as it is the most prevalent type of genetic epilepsy, but also for

pathophysiologic reasons, especially the hypothesis of epileptic networks, for which we decided to concomitantly record the EEG with TMS.

My progression towards the initial goal was hampered by two main factors. Firstly, the inclusion of people with JME was more difficult than anticipated. I contacted 15 neurologists from 15 different general hospitals in the Netherlands. Based on epidemiological studies, every neurologist should encounter 1-3 people with JME every year, depending on the size of their practice. Despite extending my inclusion period from 12 months to 18 months, only thirteen people with JME were referred to me for my study, of which eight fortunately agreed to participate. It is unclear whether the neurologists saw less people with JME than expected based on epidemiology, or whether potential participants declined participation in the study at an early stage. Secondly, after the first measurement of the control group, it became clear that the outcome measure on which the experiment was based, namely the resting motor threshold and long-interval intracortical inhibition recovery curve, showed a much larger interindividual variability than described in the literature. Exchanges with colleagues from different institutes who were working on the same subject revealed that they too, obtained variable results both in people with epilepsy and in healthy controls. Aside from my inability to reproduce the results reported in previous studies, closer examination of this literature revealed several inconsistencies, as described in **chapter 7.4.** I am of the opinion that these issues have to be resolved before TMS combined with EMG can take the next step towards implementation in the clinical practice.

9.4 Future directions

This thesis offers different insights into the interplay between cortical excitability and brain states in the context of epilepsy and migraine. But what exactly is "cortical excitability"? In **chapter 2,** I cited the definition of neuronal excitability "as the readiness of a neuron to generate an action potential when triggered, usually by an excitatory post-synaptic potential". Now, I feel that this definition may fall short of explaining *cortical* excitability and its relation to seizures. The cortex is more than a bunch of neurons with a more or less negative membrane potential. It also contains interneurons, which are functionally and anatomically connected to neurons. The cortex is connected to other brain structures, which are also involved in seizures, such as the thalamus and the brain stem. I would therefore like to coin the term

"epileptogenicity", defined as the seizure proneness of an individual. It encompasses the propensity for synchronisation (synchronisability), brain connectivity between the cortex and other structures, and also the instability of brain states that may be key to epilepsy and migraine. The clinical value of variables such as phase clustering and non-linearity to measure epileptogenicity should be addressed in future studies.

Before asking how such variables may be used, their exact pathophysiological meaning should be studied. One way to do this would be by using computational modelling. Through changing factors in the model that affect epileptogenicity and looking at the variable outcome, the relation between the variable and epileptogenicity can be studied *in silico*. If such studies provide clear answers, the value of these variables to follow-up disease activity in individual people, or to help guide treatment, can be further assessed *in vivo*. From the results shown in **chapter 8,** it appears that phase clustering may also be a way to study functional connectivity or regional changes in synchronisability, possibly by using more focused stimulation. If successfully reproduced and extended in other studies, this may help the delineation of seizure networks in focal epilepsy and reduce the need for intracranial EEG recordings. Another exciting application of TMS, which was mentioned only briefly in **chapter 2**, is its potential use to modulate properties of the brain. To date, no successful stimulation paradigms have been developed for epilepsy. Computational models may also help to address this question, and provide a useful "prescription" for a stimulation paradigm that could be used *in vivo* to treat epilepsy, or at least temporarily reduce epileptogenicity.

One of the aims of this thesis was to shed light on the relationship between migraine and epilepsy. The study in **chapter 3** has contributed to the clarification of the epidemiological association between both conditions, but the pathophysiological link remains unclear. I have shown possible signs of higher synchronisability interictally in the occipital cortex of people with migraine with visual aura in **chapter 8.** It would be interesting to study the evolution of phase clustering in the migraine cycle with TMS-EEG in people with migraine with and without aura. This may reveal changes in connectivity and phase clustering before, during, and after a migraine attack.

An unresolved issue in migraine is the occurrence of cortical spreading depression (see **chapter 2.1**). There is no direct proof of its occurrence in people. Some studies have suggested that a small band of epileptiform activity precedes the cortical depression wave (Dreier *et al.*, 2012). This may be one of the pathophysiological links between migraine and epilepsy. It was suggested that postictal depression of the EEG in epilepsy is caused by the same type of mechanism that causes spreading depression (Somjen, 2004). While both phenomena are called "depressions", based on the flattening of EEG activity, it should be kept in mind that the cellular processes underlying these phenomena may actually be the opposite. The spreading depression, possibly related to migraine aura, is likely caused by the *depolarisation* of cortical neurons (i.e. the membrane potential becomes less negative). There is evidence that after a seizure, cortical neurons become *hyperpolarised* (i.e. the membrane potential becoming *more* negative) (Somjen, 2004). Future studies are necessary to better understand the pathophysiological link between migraine and epilepsy.

9.5 Conclusion

What has this work contributed to the field of epilepsy? First, it provides additional evidence that epilepsy and migraine are related conditions. Physicians who treat people with epilepsy or migraine should be aware of this and other co-morbidities, and actively ask about symptoms related to these conditions to assure adequate treatment. Second, the insight that clonic slowing may be related to seizure termination and postictal EEG depression paves the way for further studies on how seizures terminate, but also provides a foundation for an automated warning mechanism for potentially fatal prolonged postictal states. Third, it supports the notion that JME may be a condition affecting the development of neural networks. The finding that focal EEG abnormalities may occur in up to 80% of people with JME is a stark reminder that this diagnosis can be challenging, and relies on good history taking by the treating physician. The last, and possibly most important conclusion that should be drawn from this thesis is related to TMS. I have shown that, despite its potential detailed in several studies, TMS is not ready yet to be implemented in the clinical practice to measure cortical excitability. Almost all previous studies show differences in cortical excitability parameters between people with epilepsy and healthy controls. Based on these studies, it seems only a small step to implement TMS as a clinical tool to assess cortical excitability as a biomarker in epilepsy. Through careful analysis of the literature and comparison with my own results, I have shown that many questions

remain to be resolved before this can be achieved. Perhaps the most pressing issue is that of inconsistent reports on interindividual variability, especially in the paired-pulse paradigms. A solution may be to combine TMS with EEG, which allows a more anatomically and temporally exact assessment of variables contributing to cortical excitability. I showed that phase synchrony or non-linearity may be two such variables. The ideal TMS variable is a variable that is as predictive as temperature measured with a thermometer. If it indicates a value above a certain cut-off point (38.5^{o}C for example), there is a pathological state (fever). The challenge that lies ahead is to develop a robust TMS variable that can be used to measure seizure susceptibility (epileptogenicity) on an individual basis. Only then will TMS be useful as a diagnostic tool in epilepsy.

10

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"...the majority of scholars obtain no greater reward for their painstaking work than the esteem and applause of the learned, who are a tiny minority."[10]

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Appendix 1

Electronic database search strategy – **Chapter 3.**

Ovid MEDLINE (1946-2013)

1. exp Migraine Disorders/ or exp Headache Disorders/ or exp Headache/
2. (headach* or migrain* or cephalgi* or cephalalgi*).mp.
3. 1 or 2
4. exp Epilepsy/
5. (epileps* or seizure* or convuls* or epileptic*).mp.
6. 4 or 5
7. exp Epidemiologic Methods/
8. exp Epidemiology/
9. exp Population/
10. (prevalence or incidence or epidemiolog* or population or community).mp.
11. 7 or 8 or 9 or 10
12. 3 and 6 and 11
13. animals/ not humans/
14. 12 not 13

Pubmed

(("migraine disorders"[MeSH Terms] OR "Headache"[Mesh] OR "Headache Disorders"[Mesh] OR "migrain*"[All Fields] OR "headach*"[All Fields] OR "cephalalgi*"[All Fields]) AND ("epilepsy"[MeSH Terms] OR seizur*[All Fields] OR "epilepsy"[All Fields] OR epilepsies[All Fields] OR convuls*[All Fields] OR epileptic*[All Fields]) AND (prevalence[All Fields] OR incidence[All Fields] OR epidemiolog*[All Fields] OR population[All Fields] OR community[All Fields] OR "Epidemiology"[Mesh] OR "Epidemiologic Methods"[Mesh] OR "Population"[Mesh])) NOT (("Animals"[Mesh]) NOT "Humans"[Mesh])

Ovid EMBASE (1947-2013)

1. exp headache/ or exp "headache and facial pain"/ or exp migraine/
2. (headach* or migrain* or cephalgi* or cephalalgi*).mp.
3. 1 or 2

4. exp epilepsy/
5. (epileps* or seizure* or convuls* or epileptic*).mp.
6. 4 or 5
7. exp epidemiological data/
8. exp epidemiology/
9. exp "population and population related phenomena"/
10. (prevalence or incidence or epidemiolog* or population or community).mp.
11. 7 or 8 or 9 or 10
12. 3 and 6 and 11
13. (animals/ or animal studies/) not humans/
14. 12 not 13
15. limit 14 to exclude medline journals

Web of Science (SCI & CPCI)
TS=(migraine*) AND TS=(epileps* OR seizure* OR convuls* OR epileptic*) AND TS=(prevalence OR incidence OR epidemiolog* OR population OR community)

PsycInfo
1. exp Migraine Headache/ or exp Headache/
2. (headach* or migrain* or cephalgi* or cephalalgi*).mp.
3. 1 or 2
4. exp Epilepsy/
5. (epileps* or seizure* or convuls* or epileptic*).mp.
6. 4 or 5
7. exp Epidemiology/
8. exp Population/
9. (prevalence or incidence or epidemiolog* or population or community).mp.
10. 7 or 8 or 9
11. 3 and 6 and 10
12. Animals/ not (Human Females/ or Human Males/)
13. 11 not 12

Table A1: Studies whose exclusion required greater consideration

Study	**Reason(s) for exclusion**
Ottman et al. 1994[a]	This study was not population-based. The source population did not necessarily reflect the general population but instead included participants in the Epilepsy Family Study of Columbia University, both the probands with epilepsy as well as their parents and siblings. This would potentially result in selection bias.

[a] Ottman R, Lipton RB. Comorbidity of migraine and epilepsy. *Neurology* 1994;44:2105-2110.